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| (54) Title: METHOD FOR TREATMENT OF AUTOIMMUNE DISEASES USING INTERFERON-TAU (57) Abstract Methods of treating autoimmune disorders, such as multiple sclerosis, are disclosed. The methods employ administration of interferon-tau (IFN τ) in a therapeutically-effective dose, preferably administered by oral ingestion or injection. | | |

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METHOD FOR TREATMENT OF AUTOIMMUNE DISEASES USING INTERFERON-TAU

FIELD OF THE INVENTION

The present invention relates to the use of IFN τ as a treatment for conditions
 5 relating to immune system hypersensitivity. More particularly, the present invention relates to the treatment of autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, lupus erythematosus and type I diabetes mellitus.

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BACKGROUND OF THE INVENTION

The immune system is the body's primary defense against diseases caused by invading organisms, such as bacteria, viruses or parasites, as well as diseases caused by abnormal growth of the body's own tissues (*i.e.*, cancerous tumors). Normally, the
5 immune system is able to distinguish the body's normal tissues, or self, from foreign or cancerous tissue, or non-self. The loss of recognition of a particular tissue as self, and the subsequent immune response directed against that tissue, typically results in an "autoimmune response" that often has serious clinical consequences.

One specific example of such an autoimmune disease is multiple sclerosis (MS), a
10 progressive disease of the central nervous system (CNS) in which patches of myelin (the protective covering of nerve fibers) in the brain and spinal cord are destroyed by the body's own immune system. This destruction leads to scarring and damage to the underlying nerve fibers, and may manifest itself in a variety of symptoms, depending on the parts of the
brain and spinal cord that are affected. Spinal cord damage may result in tingling or
15 numbness, as well as a heavy and/or weak feeling in the extremities. Damage in the brain may result in muscle weakness, fatigue, unsteady gait, numbness, slurred speech, impaired vision, vertigo and the like.

Current therapies for multiple sclerosis include corticosteroid drugs (to alleviate the symptoms of acute episodes), as well as other biomolecules. In particular, beta-interferon
20 (IFN β) has been tested and approved by the U.S. Food and Drug Administration (FDA) as an MS therapy. Unfortunately, the presently-used therapies suffer from a range of problems. The drugs are often toxic at the doses required for a maximal therapeutic effect. Further, the body may become desensitized to the drug such that higher (and more toxic) doses are required to maintain even a minimal therapeutic effect.

25 The present invention provides a method of treatment for autoimmune diseases, such as MS, that does not have the toxic side effects associated with currently-used therapies.

SUMMARY OF THE INVENTION

30 In one aspect, the present invention includes a method of treating an autoimmune disease in a subject in need of such treatment. In one embodiment, the autoimmune disease is multiple sclerosis. The method includes administering, to the subject, a pharmaceutically effective amount of tau-interferon. The tau-interferon may be administered, for example, orally or via intravenous or intramuscular injection. Orally-administered IFN τ is preferably

ingested by the subject. The tau interferon may be derived from (have an amino acid sequence corresponding to that of) a tau-interferon from any species that expresses tau-interferon protein (*e.g.*, ovine, bovine, goat, ox, rat, mouse or human tau-interferon).

The tau-interferon may be purified from a suitable source, produced recombinantly (*i.e.*, recombinant tau-interferon), or produced synthetically. In addition, tau-interferon polypeptides (typically having between about 15 and 172 amino acids) can be used in the method of the present invention. The method of the invention may also include administering a second autoimmune disease (*e.g.*, multiple sclerosis) treatment agent before, concurrently with, or after administering tau-interferon. Exemplary second treatment agents, or medicaments, include beta-interferon and corticosteroid drugs.

In a further embodiment, the present invention includes a method of treating lupus erythematosus in a subject in need of such treatment. The method includes administering, to the subject, a pharmaceutically effective amount of tau-interferon.

In another embodiment, the present invention includes a method of treating type I diabetes in a subject in need of such treatment. The method includes administering, to the subject, a pharmaceutically effective amount of tau-interferon.

In a further embodiment, the present invention includes a method of treating rheumatoid arthritis in a subject in need of such treatment. The method includes administering, to the subject, a pharmaceutically effective amount of tau-interferon.

The above-recited methods may also include administration by routes other than oral administration or injection, for example, topical application or intraarterial infusion. It is further contemplated that tau-interferon may be useful for treatment of either allograft or xenograft transplantation rejection.

In another aspect, the present invention includes an improvement in a method of treating a disease condition in a mammal (*e.g.*, dog or human) responsive to treatment by interferon-tau (IFN τ). The improvement comprises orally administering a therapeutically or pharmaceutically effective amount of IFN τ . The orally-administered IFN τ is preferably ingested by the mammal. In a general embodiment, the IFN τ is orally-administered at a dosage of between about 1×10^5 and about 1×10^8 units per day, preferably at a dosage of between about 1×10^6 and about 1×10^7 units per day. The IFN τ may be, for example, ovine IFN τ (OvIFN τ), *e.g.*, a polypeptide having the sequence represented as SEQ ID NO:2, or a human IFN τ (HuIFN τ), *e.g.*, a polypeptide having the sequence represented as SEQ ID NO:4 or SEQ ID NO:6.

In one embodiment, the disease condition is an immune system disorder, such as an autoimmune disorder (*e.g.*, multiple sclerosis (MS), type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, allergies or psoriasis). MS is particularly amenable to
5 treatment using the methods of the present invention.

In another embodiment, the disease condition is a cell proliferation disorder, such as a cancer (*e.g.*, hairy cell leukemia, Kaposi's Sarcoma, chronic myelogenous leukemia, multiple myeloma, superficial bladder cancer, skin cancer (basal cell carcinoma and malignant melanoma), renal cell carcinoma, ovarian cancer, low grade lymphocytic and
10 cutaneous T cell lymphoma, and glioma).

In yet another embodiment, the disease condition is a viral disease (*e.g.*, hepatitis A, hepatitis B, hepatitis C, non-A, non-B, non-C hepatitis, Epstein-Barr viral infection, HIV infection, herpes virus (EB, CML, herpes simplex), papilloma, poxvirus, picorna virus, adeno-virus, rhino virus, HTLV I, HTLV II, and human rotavirus).

15 The invention also includes a method of decreasing the severity or frequency of a relapse of multiple sclerosis (MS) in a human suffering from MS, by orally administering a therapeutically or pharmaceutically effective amount of interferon-tau (IFN τ) to the human. Examples of dosages and sources of IFN τ are as presented above.

In another aspect, the invention includes a method of treating a cell proliferation
20 disorder in a subject, by orally administering a therapeutically or pharmaceutically effective amount of interferon-tau (IFN τ) to the subject. The orally-administered IFN τ is preferably ingested by the subject. Examples of cell proliferation disorders amenable to treatment, dosages, and sources of IFN τ are as presented above.

In still another aspect, the invention includes a method of treating a viral disease in
25 a subject, by orally administering a therapeutically or pharmaceutically effective amount of interferon-tau (IFN τ) to the subject. The orally-administered IFN τ is preferably ingested by the subject. Examples of viral diseases amenable to treatment, dosages, and sources of IFN τ are as presented above.

A further aspect of the invention includes a method of enhancing fertility in a
30 female mammal (*e.g.*, dog or human), by orally administering a therapeutically or pharmaceutically effective amount of interferon-tau (IFN τ) to the mammal. Examples of dosages and sources of IFN τ are as presented above.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

5 **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows a comparison of the toxicity of IFN β and IFN γ .

Figure 2 shows the mean severity of experimental allergic encephalomyelitis (EAE) in New Zealand White (NZW) mice immunized with MBP in the presence and absence of IFN γ .

10 Figure 3 shows the effects of IFN γ on proliferation of spleen cells from MBP-immunized NZW mice.

Figures 4A, 4B, 4C, 4D, 4E and 4F are graphic depictions of superantigen reactivation of EAE in the presence and absence of IFN γ .

Figure 5 shows the effects of IFN γ on V β -specific T-cell activation.

15 Figure 6 shows the amount of OvIFN γ in NZW mouse sera after administration by either oral feeding (filled bars) or i.p. injection (open bars) as measured using an anti-viral assay.

Figures 7A, 7B and 7C show the prevention of chronic-relapsing experimental allergic encephalomyelitis (EAE) in SJL mice by orally-administered (Fig. 7C) and i.p.-
20 injected (Fig. 7B) IFN γ as compared with mice receiving no treatment (Fig. 7A).

Figures 8A, 8B and 8C show sections of mouse spinal cord stained with cresyl violet for detection of lymphocyte infiltration from EAE-induced animals receiving either no IFN γ treatment (Fig. 8A), OvIFN γ treatment by i.p. injection (Fig. 8B) or OvIFN γ treatment by oral feeding (Fig. 8C).

25 Figure 9 shows induction of IL-10 by either single-dose or prolonged IFN γ treatment administered by i.p. injection or oral feeding.

Figure 10 shows relapses of EAE in SJL mice following removal of IFN γ treatment.

Figure 11 shows ELISA detection of anti-OvIFN γ antibodies in the sera of OvIFN γ -
30 treated mice following i.p. injection or oral feeding of OvIFN γ .

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is the nucleotide sequence of a synthetic gene encoding ovine interferon- γ (OvIFN γ). Also shown is the encoded amino acid sequence.

SEQ ID NO:2 is an amino acid sequence of a mature OvIFN γ protein.

SEQ ID NO:3 is a synthetic nucleotide sequence encoding a mature human interferon- γ (HuIFN γ) protein.

SEQ ID NO:4 is an amino acid sequence for a mature HuIFN γ 1 protein.

5 SEQ ID NO:5 is the nucleotide sequence, excluding leader sequence, of genomic DNA clone HuIFN γ 3, a natural HuIFN γ gene.

SEQ ID NO:6 is the predicted amino acid sequence of a mature human IFN γ protein encoded by HuIFN γ 3, encoded by the sequence represented as SEQ ID NO:5.

10 DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Interferon- γ refers to any one of a family of interferon proteins having at least one characteristic from each of the following two groups of characteristics: (i) (a) anti-luteolytic properties; (b) anti-viral properties; (c) anti-cellular proliferation properties; and (ii) about

15 45 to 68% amino acid homology with α -Interferons and greater than 70% amino acid homology to known IFN γ sequences (*e.g.*, Ott, *et al.*, 1991; Helmer, *et al.*, 1987; Imakawa, *et al.*, 1989; Whaley, *et al.*, 1994; Bazer, *et al.*, 1994). Amino acid homology can be determined using, for example, the LALIGN program with default parameters. This program is found in the FASTA version 1.7 suite of sequence comparison programs
20 (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA). IFN γ can be obtained from a number of sources including cows, sheep, ox, and humans.

An *interferon- γ polypeptide* is a polypeptide having between about 15 and 172 amino acids derived from an interferon- γ amino acid coding sequence, where said 15 to 172
25 amino acids are contiguous in native interferon- γ . Such 15-172 amino acid regions can also be assembled into polypeptides where two or more such interferon- γ regions are joined that are normally discontinuous in the native protein.

Treating a disease refers to administering a therapeutic substance effective to reduce the symptoms of the disease and/or lessen the severity of the disease.

30

II. Overview of Invention

Experiments performed in support of the present invention indicate that IFN γ is effective at preventing the development of experimental allergic encephalomyelitis (EAE; Zamvil and Steinman, 1990), an animal model of antigen-induced autoimmunity that has

been widely studied to gain insight into multiple sclerosis (MS). IFN γ is at least as effective in these experiments as IFN β , which has recently been approved by the FDA for the treatment of MS. The experiments further show that IFN γ has a lower toxicity than IFN β , and that IFN γ -treated mice do not develop leukopenia, an undesired side effect associated with IFN β treatment.

In addition, experiments performed in support of the present invention have demonstrated that orally-administered IFN γ is nearly as effective as injected IFN γ at treating EAE, but results in significantly lower anti-IFN γ antibody titers in the treated individuals. This unexpected advantage results in a decreased chance of side-effects due to a host immune response against IFN γ .

It has recently been shown that superantigens can include relapses in EAE, similar to those that occur "spontaneously" in MS patients. Additional experiments performed in support of the present invention show that IFN γ blocks superantigen reactivation of EAE, and that the inhibitory effect of IFN γ on induction of EAE and reactivation by superantigen involves suppression of myelin basic protein (MBP) and superantigen activation of T cells as well as suppressed induction of destructive cytokines such as tumor necrosis factor. Taken together, these results indicate that IFN γ , both injected and orally-administered, may be highly effective in treatment of autoimmune diseases, such as MS, with lower toxicity and fewer side effects than are associated with IFN β .

20

III. Interferon- γ

The first IFN γ to be identified was ovine IFN γ (OvIFN γ). Several isoforms of the 18-19 kDa protein were identified in conceptus (the embryo and surrounding membranes) homogenates (Martal, *et al.*, 1979). Subsequently, a low molecular weight protein released into conceptus culture medium was purified and shown to be both heat labile and susceptible to proteases (Godkin, *et al.*, 1982). OvIFN γ was originally called ovine trophoblast protein-one (oTP-1) because it was the primary secretory protein initially produced by trophoblast of the sheep conceptus during the critical period of maternal recognition in sheep. Subsequent experiments have determined that OvIFN γ is a pregnancy recognition hormone essential for establishment of the physiological response to pregnancy in ruminants, such as sheep and cows (Bazer and Johnson, 1991).

30

IFN γ s with similar characteristics and activities have been isolated from other ruminant species including cows and goats (Bartol, *et al.*, 1985; and Gnatek, *et al.*, 1989). Antisera to all the IFN γ s cross-react. This is not unexpected since the species specific

forms of IFN τ are more closely homologous to each other than to the IFN α from the identical species (Roberts, *et al.*, 1992).

The cow protein (BoIFN τ ; Helmer, *et al.*, 1987; Imakawa, *et al.*, 1989) has similar functions to OvIFN τ in maternal recognition of pregnancy. Further, it shares a high degree of amino acid and nucleotide sequence homology with OvIFN τ . The nucleic acid sequence homology between OvIFN τ and BoIFN τ is 76.3% for the 5' non-coding region, 89.7% for the coding region, and 91.9% for the 3' non-coding region. The amino acid sequence homology is 80.4%.

An IFN τ cDNA obtained by probing a sheep blastocyst library with a synthetic oligonucleotide representing the N-terminal amino acid sequence (Imakawa, *et al.*, 1987) has a predicted amino acid sequence that is 45-55% homologous with IFN α from human, mouse, rat and pig and 70% homologous with bovine IFN α II, now referred to as IFN Ω . Several cDNA sequences have been reported which may represent different isoforms (Stewart, *et al.*, 1989; Klemann, *et al.*, 1990; and Charlier, M., *et al.*, 1991). All are approximately 1 kb with a 585 base open reading frame that codes for a 23 amino acid leader sequence and a 172 amino acid mature protein. The predicted structure of IFN τ as a four helical bundle with the amino and carboxyl-termini in apposition further supports its classification as a type I IFN (Jarpe, *et al.*, 1994).

TABLE 1

OVERVIEW OF THE INTERFERONS

| Aspects | Type I | | | Type II |
|--------------------|---------------------|------------|-------------|------------|
| Types | α & ω | β | τ | γ |
| Produced by: | leukocyte | fibroblast | trophoblast | lymphocyte |
| Effects: | | | | |
| Antiviral | + | + | + | + |
| Antiproliferative | + | + | + | + |
| Pregnancy Signally | - | - | + | - |

While IFN τ displays many of the activities classically associated with type I IFNs (see Table 1, above), considerable differences exist between it and the other type I IFNs. The most prominent difference is its role in pregnancy, detailed above. Also different is viral induction. All type I IFNs, except IFN τ , are induced readily by virus and dsRNA

(Roberts, *et al.*, 1992). Induced IFN α and IFN β expression is transient, lasting approximately a few hours. In contrast, IFN γ synthesis, once induced, is maintained over a period of days (Godkin, *et al.*, 1982). On a per-cell basis, 300-fold more IFN γ is produced than other type I IFNs (Cross and Roberts, 1991).

5 Other differences may exist in the regulatory regions of the IFN γ gene. For example, transfection of the human trophoblast cell line JAR with the gene for bovine IFN γ resulted in antiviral activity while transfection with the bovine IFN Ω gene did not. This implies unique transacting factors involved in IFN γ gene expression. Consistent with this is the observation that while the proximal promoter region (from 126 to the transcriptional

10 start site) of IFN γ is highly homologous to that of IFN α and IFN β ; the region from -126 to -450 is not homologous and enhances only IFN γ expression (Cross and Roberts, 1991). Thus, different regulatory factors appear to be involved in IFN γ expression as compared with the other type I IFNs.

IFN γ expression may also differ between species. For example, although IFN γ

15 expression is restricted to a particular stage (primarily days 13-21) of conceptus development in ruminants (Godkin, *et al.*, 1982), preliminary studies suggest that the human form of IFN γ is constitutively expressed throughout pregnancy (Whaley, *et al.*, 1994).

A. Isolation of IFN γ

20 OvIFN γ protein may be isolated from conceptuses collected from pregnant sheep and cultured *in vitro* in a modified Minimum Essential Medium (MEM) as described by Godkin, *et al.*, (1982) and Vallet, *et al.*, (1987). The IFN γ may be purified from the conceptus cultures by ion exchange chromatography and gel filtration. The homogeneity of isolated IFN γ may be assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis

25 (SDS-PAGE; Maniatis, *et al.*, 1982; Ausubel, *et al.*, 1988), and determination of protein concentration in purified IFN γ samples may be performed using the bicinchoninic (BCA) assay (Pierce Chemical Co., Rockford, IL; Smith, *et al.*, 1985).

B. Recombinant Production of IFN γ

30 Recombinant IFN γ protein may be produced from any selected IFN γ polynucleotide fragment using a suitable expression system, such as bacterial or yeast cells. The isolation of IFN γ nucleotide and polypeptide sequences is described in Bazer, *et al.* (1994). For example, Bazer, *et al.*, describe the identification and isolation of a human IFN γ gene. A synthetic nucleotide sequence encoding a mature human interferon- γ (HuIFN γ) protein is

presented herein as SEQ ID NO:3. SEQ ID NO:4 is the corresponding amino acid sequence for a mature HuIFN γ 1 protein. SEQ ID NO:5 is the nucleotide sequence, excluding leader sequence, of genomic DNA clone HuIFN γ 3, a natural HuIFN γ gene, and SEQ ID NO:6 is the predicted amino acid sequence of a mature human IFN γ protein encoded by the sequence represented as SEQ ID NO:5.

To make an IFN γ expression vector, an IFN γ coding sequence (*e.g.*, SEQ ID NO:1) is placed in an expression vector, *e.g.*, a bacterial expression vector, and expressed according to standard methods. Examples of suitable vectors include lambda gt11 (Promega, Madison WI); pGEX (Smith, *et al.*, 1985); pGEMEX (Promega); and pBS (Stratagene, La Jolla CA) vectors. Other bacterial expression vectors containing suitable promoters, such as the T7 RNA polymerase promoter or the tac promoter, may also be used. Cloning of the OvIFN γ synthetic polynucleotide into a modified pIN III omp-A expression vector is described in the Materials and Methods.

For the experiments described herein, the OvIFN γ coding sequence present in SEQ ID NO:1 was cloned into a vector, suitable for transformation of yeast cells, containing the methanol-regulated alcohol oxidase (AOX) promoter and a Pho1 signal sequence. The vector was used to transform *P. pastoris* host cells and transformed cells were used to express the protein according to the manufacturer's instructions (Invitrogen, San Diego, CA).

Other yeast vectors suitable for expressing IFN γ for use with methods of the present invention include 2 micron plasmid vectors (Ludwig, *et al.*, 1993), yeast integrating plasmids (YIps; *e.g.*, Shaw, *et al.*, 1988), YEP vectors (Shen, *et al.*, 1986), yeast centromere plasmids (YCps; *e.g.*, Ernst, 1986), and other vectors with regulatable expression (Hitzeman, *et al.*, 1988; Rutter, *et al.*, 1988; Oeda, *et al.*, 1988). Preferably, the vectors include an expression cassette containing an effective yeast promoter, such as the MF α 1 promoter (Ernst, 1986; Bayne, *et al.*, 1988, GADPH promoter (glyceraldehyde-3-phosphate-dehydrogenase; Wu, *et al.*, 1991) or the galactose-inducible GAL10 promoter (Ludwig, *et al.*, 1993; Feher, *et al.*, 1989; Shen, *et al.*, 1986). The yeast transformation host is typically *Saccharomyces cerevisiae*, however, as illustrated above, other yeast suitable for transformation can be used as well (*e.g.*, *Schizosaccharomyces pombe*, *Pichia pastoris* and the like).

Further, a DNA encoding an IFN γ polypeptide can be cloned into any number of commercially available vectors to generate expression of the polypeptide in the appropriate host system. These systems include the above described bacterial and yeast expression

systems as well as the following: baculovirus expression (Reilly, *et al.*, 1992; Beames, *et al.*, 1991; Clontech, Palo Alto CA); plant cell expression, transgenic plant expression (*e.g.*, Gelvin and Schilperoot), and expression in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL, Gaithersburg MD). The recombinant polypeptides can be expressed as fusion proteins or as native proteins. A number of features can be engineered into the expression vectors, such as leader sequences which promote the secretion of the expressed sequences into culture medium. The recombinantly produced polypeptides are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, and affinity chromatography. Immunoaffinity chromatography can be employed, as described above, using antibodies generated based on the IFN γ polypeptides.

In addition to recombinant methods, IFN γ proteins or polypeptides can be isolated from selected cells by affinity-based methods, such as by using appropriate antibodies.

Further, IFN γ peptides may be chemically synthesized using methods known to those skilled in the art.

C. IFN γ Lacks Toxicity

Type I IFNs (IFN α and IFN β), as well as type II (IFN γ), exhibit significant cytotoxicity (Degre, 1974; Fent and Zbinden, 1987). Detrimental toxic effects exerted by these IFNs have been observed during clinical trials and patient treatment, and include flu-like symptoms such as fever, chills and lethargy, tachycardia, nausea, weight loss, leukopenia, and neutropenia (Degre, 1974; Fent and Zbinden, 1987).

Experiments performed in support of the present invention and detailed in Example 1, below, suggest that IFN γ has significantly lower cytotoxicity than the IFNs listed above. Cytotoxicity was assessed *in vivo* (Example 1A) using white blood cell counts (WBC), lymphocytes percentages and total body weights of New Zealand White (NZW) mice injected with the various IFNs. The results are presented in Table 3 and summarized in Table 2a. Twelve hours after injection with 10^5 U of murine interferon-alpha (MuIFN α), shown previously to induce a higher degree of toxicity than IFN β , the mice exhibited decreased white blood cell counts, lymphopenia and substantial weight loss. None of these toxicity-related effects were observed in OvIFN γ -injected animals. The concentrations of OvIFN γ used in the toxicity studies were the same as those shown to be effective at preventing EAE (detailed in Example 2, below).

Cytotoxicity was also assessed *in vitro* (Example 1B). Viability of L929 cells exposed IFN γ at concentrations as high as 200,000 U/ml remained near control levels, while IFN β showed toxic effects at concentrations as low as 7,000 U/ml (Figure 1). IFN γ was also found to lack toxicity when tested in a panel of tumorigenic cell lines, although it did inhibit cell replication. The results of these and additional studies, comparing the toxicity of IFN γ with the toxicities of IFN β and IFN α in animal models as well as tissue culture (Bazer and Johnson, 1991; Johnson, *et al.*, 1994; Bazer, *et al.*, 1989; and Soos and Johnson, 1995), are summarized in Table 2a, below.

TABLE 2A

PARAMETERS DEMONSTRATING THE LACK OF TOXICITY
BY IFN γ BUT NOT IFNS α AND β

| Toxicity | | | |
|---|--------------|--------------|-------------|
| <i>In vitro</i> (cell viability) | IFN γ | IFN α | IFN β |
| Mouse L929 (50,000-200,000 U/ml of IFN) | - | + | + |
| Bovine MDBK (50,000 U/ml of IFN) | - | + | ND |
| Human WISH (50,000 U/ml of IFN) | - | + | ND |
| Human Peripheral Lymphocytes (50,000 U/ml of IFN) | - | + | + |
| HIV Infected Human Peripheral Lymphocytes (50,000-500,000 U/ml of IFN) | - | + | ND |
| <i>In vivo</i> (NZW Mice) | IFN γ | IFN α | IFN β |
| White Blood Cell Count | - | + | + |
| Lymphocyte Depression | - | + | + |
| Weight Measurement | - | + | \pm |
| Plus and minus signs indicate toxicity or lack thereof induced by treatment with the various type I IFNs. For <i>in vivo</i> studies, 10 ⁵ U were administered per injection and cell counts and weights were evaluated at either 12 or 24 hours after injection. ND = not determined. | | | |

MDBK cells cultured in the presence of IFNs exhibited reduced viability when cultured in the presence of IFN α (50,000 U/ml), but not when cultured in the presence of IFN τ (Pontzer, *et al.*, 1991). Similar results were obtained with the human WISH cell line. Comparisons of toxicity (or lack thereof) induced by IFN τ and other IFNs have been made using human peripheral mononuclear cells (HPMC) and HIV-infected HPMC. IFN τ did not exhibit toxic effects on cultured HPMC while both IFN α and IFN β reduced cell viability at 50,000 U/ml (Soos and Johnson, 1995). Human lymphocytes infected with HIV-1 and feline lymphocytes infected with HIV also did not exhibit reduced viability in the presence of IFN τ (Bazer, *et al.*, 1989). These findings indicate that the lack of toxicity of IFN τ inferred from observations using immortalized cell lines also applies to human peripheral blood. The results summarized in Table 2a demonstrate that injected IFN τ appears to have little or no toxicity, when tested both *in vitro* and *in vivo*, as compared with injected IFN α , IFN β and IFN γ .

Additional experiments performed in support of the present invention compared the toxicity, measured by lymphocyte depression in peripheral blood, of orally-administered and injected OvIFN τ with that of orally-administered and injected IFNs α and β . Blood was obtained from the tail and white blood cells (WBC) counts were enumerated using a hemocytometer. Differential WBC counts were performed on Wright-Giemsa-stained blood smears.

The results are shown in Tables 2b, 2c and 2d, below. Significant levels of toxicity were detected in mice fed either IFN α and β while no significant lymphocyte depression was detected in mice fed 10^5 , 2×10^5 or 5×10^5 U of OvIFN τ or PBS alone. These data suggest that orally-administered OvIFN τ (like injected OvIFN τ) has significantly-reduced toxicity with respect to other type I IFNs.

Tables 2b-2d

Comparison of IFNs τ , β and α for Toxicity After Oral Feeding

Table 2b

| IFN (DOSE) | CELL COUNT (CELL NO. $\times 10^3$) | |
|------------|--------------------------------------|---------------|
| | BEFORE ORAL FEEDING | |
| | TOTAL WBC | LYMPHOCYTES |
| PBS | 7.0 ± 1.4 | 6.1 ± 1.2 |

| | | |
|------------------------|---------------|---------------|
| $\tau(10^5)$ | 7.5 ± 0.7 | 6.4 ± 0.6 |
| $\tau(2 \times 10^5)$ | 6.5 ± 0.7 | 5.3 ± 0.6 |
| $\tau(5 \times 10^5)$ | 7.5 ± 0.7 | 6.5 ± 0.6 |
| $\beta(10^5)$ | 7.0 ± 0.7 | 5.9 ± 1.2 |
| $\beta(2 \times 10^5)$ | 7.5 ± 2.1 | 6.5 ± 1.8 |
| $\alpha(10^5)$ | 7.5 ± 0.7 | 6.6 ± 0.6 |

Table 2c

| IFN (DOSE) | CELL COUNT (CELL NO. $\times 10^3$) | | |
|------------------------|--------------------------------------|-----------------|-------------------------|
| | 18 H AFTER ORAL FEEDING | | |
| | TOTAL WBC | LYMPHOCYTES | % LYMPHOCYTE DEPRESSION |
| PBS | -- | -- | -- |
| $\tau(10^5)$ | 7.0 ± 1.4 | 6.0 ± 1.3 | 6.2 |
| $\tau(2 \times 10^5)$ | 7.0 ± 2.8 | 5.9 ± 2.4 | 0 |
| $\tau(5 \times 10^5)$ | 7.5 ± 2.1 | 6.3 ± 1.8 | 3.1 |
| $\beta(10^5)$ | 6.5 ± 0.7 | 5.1 ± 0.6 | 13.6 |
| $\beta(2 \times 10^5)$ | 6.5 ± 0.7 | $4.1 \pm 0.4^*$ | 37.0 |
| $\alpha(10^5)$ | 6.5 ± 2.1 | 4.7 ± 1.6 | 28.8 |

* $p < 0.05$ **Table 2d**

| IFN (DOSE) | CELL COUNT (CELL NO. $\times 10^3$) | | |
|-----------------------|--------------------------------------|---------------|-------------------------|
| | 24 H AFTER ORAL FEEDING | | |
| | TOTAL WBC | LYMPHOCYTES | % LYMPHOCYTE DEPRESSION |
| PBS | 7.5 ± 0.7 | 6.4 ± 0.6 | 0 |
| $\tau(10^5)$ | 8.0 ± 2.8 | 6.9 ± 2.4 | 0 |
| $\tau(2 \times 10^5)$ | 7.0 ± 1.4 | 6.0 ± 1.1 | 0 |
| $\tau(5 \times 10^5)$ | 8.0 ± 4.2 | 7.0 ± 3.6 | 0 |

| | | | |
|------------------------|---------------|-------------------------|------|
| $\beta(10^5)$ | 6.5 ± 3.5 | 5.1 ± 2.8 | 13.6 |
| $\beta(2 \times 10^5)$ | 6.5 ± 0.7 | $4.0 \pm 0.4^{\dagger}$ | 38.5 |
| $\alpha(10^5)$ | 7.0 ± 0 | $5.0 \pm 0^{\ddagger}$ | 24.2 |

$^{\dagger}p < 0.05$

$^{\ddagger}p < 0.03$

IV. IFN γ as a Treatment for Autoimmune Disorders

Compositions and methods of the present invention may be used to therapeutically treat and thereby alleviate a variety of immune system-related disorders characterized by hyper- or hypo-active immune system function. Such disorders include hyperallergenicity and autoimmune disorders, such as multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, allergies, psoriasis and the like.

A. IFN γ treatment in EAE, an Animal Model for Multiple Sclerosis

1. OvIFN γ Inhibits Development of EAE, an Animal Model for Multiple Sclerosis. The efficacy of IFN γ in treating autoimmune disorders may be evaluated in rodents with experimental allergic encephalomyelitis (EAE), an animal model of antigen-induced autoimmunity that is widely studied to gain insight into human multiple sclerosis (MS). EAE is an autoimmune demyelinating disease induced by immunizing susceptible mouse, rat or guinea pig strains with myelin basic protein (MBP) or with encephalitogenic peptide fragments. Genetic susceptibility in the model animal strains is based in part on the capacity of encephalitogenic peptides to bind to particular class II major histocompatibility complex (MHC-II) molecules (Fritz, *et al.*, 1983; Wraith, *et al.*, 1989). In particular, mice having the H-2^d haplotype are susceptible to EAE. Susceptible mouse strains include PL/J mice (Klein, *et al.*, 1983), (PL/J \times SJL)F₁ mice (Zamvil, *et al.*, 1990; Wraith, *et al.*), B10.PL mice (Figuro, *et al.*, 1982), NZW mice (Kotzin, *et al.*, 1987), and (NZB \times NZW)F₁ (Kotzin, *et al.*) mice.

Gamma-interferon (IFN γ) and beta-interferon (IFN β) have been demonstrated to be effective in treating multiple sclerosis (Johnson, *et al.*, 1994; IFN β Multiple Sclerosis Study Group, 1993). In fact, IFN β has been approved by the FDA as a therapeutic for multiple sclerosis. Although β -IFN is effective against MS, it has relatively high toxicity, and as a result, has a variety of undesirable side effects. As described above, however, IFN γ has

significantly lower toxicity than other interferons and may therefore exhibit fewer undesirable side effects.

In experiments performed in support of the present invention and detailed in Example 2, IFN- τ was tested for its ability to prevent the induction of EAE. EAE was induced in New Zealand White (NZW) mice by immunization with bovine myelin basic protein (bMBP). The mice were injected intraperitoneally (i.p.) with either a single dose of recombinant ovine IFN-tau (OvIFN τ) or murine IFN-beta (MuIFN- β) on the day of, or 3 doses of OvIFN- τ or MuIFN- β 48 hours before, on the day of and 48 hours after immunization with MBP.

The results of the experiments are summarized in Table 4. A time course of the mean severity of EAE is presented in Figure 2. Symbols are as follows: Δ - control animal; \oplus - single dose of OvIFN τ ; \square - 3 doses of OvIFN τ .

All of the animals injected (both sham-injected and IFN-injected) on the day of the immunization developed EAE, but the severity was reduced, and the mean day of onset was delayed in both the OvIFN τ (23.8 ± 0.5 days) and MuIFN- β (23.5 ± 0.6 days) treated animals relative to control animals (16.2 ± 0.8 days).

The results obtained using the 3-dose protocol are more striking. Seven of the nine control animals developed EAE an average of 15.2 days following immunization. In contrast, none of nine animals treated with OvIFN τ developed the disease, and one of nine animals treated with MuIFN- β succumbed to EAE (22 days after immunization).

The data demonstrate that IFN τ is an effective immunotherapy for the prevention of EAE, and is as effective a treatment in this model of autoimmune disease as MuIFN β . Taken together with the lower toxicity of IFN τ relative to IFN β , the data suggest that treatment of individuals having an autoimmune disorder (such as multiple sclerosis) with IFN τ may be preferable and more effective than treatment with IFN β .

2. OvIFN τ Inhibits T-Cell Proliferation. The effects of IFN τ on proliferation of spleen cells from MBP-immunized NZW mice stimulated with MBP *in vitro* were assessed. The results are shown in Figure 3. Proliferation in response to MBP was vigorous and could be reduced by IFN τ in a dose-dependent manner, indicating that IFN τ has antiproliferative activity against T cells specific for the autoantigen, MBP. These results are consistent with the observation that IFN τ inhibits or eliminates symptoms of MBP-induced EAE, since inhibition of such T-cells would be expected to reduce the severity of the autoimmune response.

3. OvIFN γ Inhibits Superantigen Reactivation of EAE. The

symptomology of MS can often be observed to occur in a relapsing-remitting manner. This form of MS consists of presentation with clinical symptoms of MS followed by periods of remission. How relapses and exacerbations occur and what causes the reactivation of

5. autoimmune disease has been a topic of much speculation. It has been suggested that environmental influences may contribute to or even be responsible for exacerbations of autoimmune disease. Such influences potentially include exposure to infectious agents as well as factors possessing immunostimulatory activity. One class of proteins which are ubiquitous in our environment are the microbial superantigens.

- 10 Microbial superantigens are toxins produced by a variety of bacteria, viruses, and other organisms such as mycoplasma that possess extremely potent immunostimulatory activity (Langford, *et al.*, 1978; Carlsson and Sjogren, 1985; and Johnson and Magazine, 1988). They are responsible for a number of maladies including food poisoning and toxic shock syndrome (Bergdoll, *et al.*, 1981). Such powerful immunostimulation by superanti-
15 gens is based on their ability to engage major histocompatibility complex class II molecules and then, as a binary complex, bind to the T cell receptor in a β -chain variable region (V β)-specific manner (Johnson, *et al.*, 1991; Janeway, *et al.*, 1989; White, *et al.*, 1989; Carlsson, *et al.*, 1988; and Fleischer and Schrezenmeier, 1988). This binding triggers T cell activation leading to proliferation of as much as 20% of a T cell repertoire (Johnson, *et al.*, 1991).

- 20 Superantigen-induced T cell proliferation is accompanied by massive amounts of cytokine production including interleukin 2 (IL2), IFN γ , and tumor necrosis factor alpha (TNF α). Of the cytokines whose production is induced by superantigen stimulation, IFN γ and TNF α have been implicated as mediators of autoimmune pathogenesis. IFN γ has been
25 shown to cause exacerbations of MS in clinical trials (Panitch, *et al.*, 1987a; Panitch, *et al.*, 1987b). Production of TNF α has been shown to be a requirement for the encephalitogenicity of certain T cell lines used to adoptively transfer EAE (Powell, *et al.*, 1990) as well as causing myelin producing oligodendrocyte death *in vitro* (Selmaj and Raine, 1988).

- 30 Experiment performed in support of the present invention that *Staphylococcus* Enterotoxin B (SEB) -induced cytokine production is also altered by IFN γ . Spleen cells from MBP-immunized mice were stimulated with SEB *in vitro* in the presence or absence of IFN γ , and supernatants were examined for TNF α and IFN γ production. Addition of IFN γ to cultures stimulated with SEB significantly reduced production of both TNF α and IFN γ .

In view of the above, these results are consistent with the ability of IFN γ to reduce the severity of EAE, and suggest that IFN γ may reduce exacerbations of MS.

Exacerbation evidenced as a clinical relapse of EAE was first demonstrated by the administration of a microbial superantigen. In the PL/J strain, acute episodes of EAE usually resolve and clinical relapses have been shown not to occur (Fritz, *et al.*, 1983). After resolution of all clinical signs of EAE induced by immunization with MBP, administration of either of the *Staphylococcus aureus* enterotoxin (SE) superantigens, SEB or

Staphylococcus Enterotoxin A (SEA), was shown to cause reactivation of disease (Schiffenbauer, *et al.*, 1993). Multiple episodes of disease exacerbation over a four-month period were also shown in which EAE could be reactivated and resolved based on multiple injections of SEB (Schiffenbauer, *et al.*, 1993). Reactivation of EAE by SEB has also been shown to occur in other susceptible strains including NZW. SEB can also reactivate disease when an acetylated amino terminal peptide of MBP is employed as the immunogen (Brocke, *et al.*, 1993).

In addition to reactivation of EAE, SEB can also prevent EAE when administered prior to immunization with MBP (Soos, *et al.*, 1993; and Kalman, *et al.*, 1993). Anergy and/or deletion of the V β 8⁺ T cell subset which is responsible for the initial induction of EAE appears to be the mechanism for this protection. Targeting of a V β specific T cell population does not, however, provide absolute protection from development of EAE. When mice protected from development of EAE by SEB pre-treatment are exposed to SEA (which has a different V β T cell specificity from SEB), induction of EAE does occur. This SEA-induced EAE is characterized by severe paralysis and accelerated onset of clinical symptoms. Thus, the effects of microbial superantigens introduce a profound complexity to autoimmune disease models such as EAE, akin to the complexity of the pathogenesis observed in MS.

The effect of OvIFN γ treatment on exacerbations of EAE induced by superantigen is evaluated on NZW mice in Example 4. The studies have also been carried out on PL/J mice. Treatment with OvIFN γ when administered in 3 doses of 10⁵ U (48 hours prior to SEB injection, on the day of SEB injection and 48 hours after SEB injection) blocked EAE reactivation by superantigen. In comparison, untreated control groups exhibited superantigen reactivation of EAE consistent with previous studies (Schiffenbauer, *et al.*, 1993).

The observation that OvIFN γ can block superantigen-induced exacerbations of EAE may be a corollary to the reduction in disease exacerbations in MS patients undergoing treatment with IFN β 1b. A summary of the studies showing that OvIFN γ can prevent

development and superantigen reactivation of EAE is presented in Table 5. The results demonstrate that IFN γ can also modulate the effects of environmental factors on the course of autoimmune disease, such as MS.

Additional experiments performed in support of the present invention have further shown that a second immunization of MBP can not reactivate EAE, and that injection of superantigens can induce an initial episode of clinical disease in PL/J mice that had been immunized with MPB but did not develop EAE. The experiments further demonstrate that this induction can be blocked by treatment with IFN γ , and that IFN γ can block superantigen-induced exacerbations of EAE akin to the reduced exacerbations of disease observed in IFN β 1b treated MS patients.

4. IFN γ Inhibits V β -specific T-Cell Activation. The effect of IFN γ treatment of SEB-induced V β specific T cell expansion *in vitro* was evaluated as described in Example 5. V β specific T-cell FACS analysis was performed on naive, SEB-injected, or IFN γ and SEB -injected NZW mice. Analyses were performed 72 hours after the injections.

Results of exemplary experiments are shown in Figure 5. Open bars represent naive animals; closed bars represent SEB-injected animals, and crosshatched bars represent IFN γ - and SEB-injected animals. Naive NZW mice exhibited 5.1 ± 0.1 % V β 8⁺CD4⁺ T cells, which was expanded to 10.2 ± 0.2 % after injection of SEB. When an IFN γ injection preceded the SEB injection, expansion of the V β 8⁺CD4⁺ T-subset was limited to 7.6 ± 0.2 %. Partial inhibition of V β 7⁺ and V β 11⁺ T cells, for which SEB is also specific, was also observed.

These data indicate that treatment with IFN γ can partially inhibit SEB-induced V β T cell expansion *in vivo*, and further support the observation that IFN γ reduces the severity of MBP-induced EAE.

B. Other Autoimmune Disease Models

In addition to EAE, other animal models of autoimmune disease may be used to evaluate the therapeutic effects of IFN γ . For example, certain strains of mice are particularly susceptible to murine systemic lupus erythematosus, a disease analogous to systemic lupus erythematosus in humans. In particular, the MRL-*lpr/lpr* lupus mouse (Singer, *et al.*, 1986) exhibits many of the same immunological characteristics of human systemic lupus erythematosus. The animals have lymphoid organ enlargement and increased T-cell

X

proliferation, with V_{β} gene expression significantly skewed in favor of $V_{\beta 8.2/8.3}$ genes (Singer, *et al.*).

MRL-*lpr/lpr* mice may be obtained from the Jackson Laboratory (Bar Harbor, ME). The onset of disease in the MRL-*lpr/lpr* mice is spontaneous (at about 3 months of age), so the disease does not need to be induced as it does in the case of EAE. To evaluate the effects of IFN γ on the progression of disease, the animals are treated with injections of IFN γ (*e.g.*, as described above) at selected intervals (*e.g.*, once every two weeks) starting at a selected age (*e.g.*, 6 weeks of age) for a selected duration (*e.g.*, until 6 months of age).

The effects of the therapy may be evaluated in several ways. For example, the relative number of $V_{\beta 8}^{+}$ cells in spleens and lymph nodes of treated and untreated groups of animals may be determined using FACS analysis as described above. An effective dose of IFN γ results in a significant reduction of the number of $V_{\beta 8}^{+}$ T cells. Further, the physical symptoms of the disease (lymphoid hyperplasia, necrosis of ear, hair loss) may be quantitated (Kim, *et al.*, 1991) and compared between treated and untreated groups. The animals may also be assayed for the reduction of ds-DNA-specific antibody and/or reduction in nephritis with proteinuria, for example, as described in Kim, *et al.*, following treatment with IFN γ .

Another animal model of an autoimmune disorder, which may be employed to evaluate the therapeutic effects of IFN γ , is adjuvant-induced arthritis in dogs (Kaplan, *et al.*, 1993).

V. Effectiveness of Orally-Administered IFN γ

Experiments performed in support of the present invention demonstrate that orally-administered IFN γ polypeptide compositions are comparable in efficacy to injected IFN γ compositions with respect to the treatment of diseases or disease conditions which benefit from treatment with IFN γ , such as autoimmune diseases (*e.g.*, multiple sclerosis).

As discussed below, not only was orally-administered IFN γ effective at treating a disease benefiting from IFN γ treatment (EAE), but the oral route of administration resulted in unexpected advantages relative to treatment with injected IFN γ compositions. For example, orally-administered IFN γ resulted in a significantly lower level of anti-IFN γ antibodies in the serum of treated individuals (see Example 12). This is beneficial because the orally-administered IFN γ is therefore less likely to be rendered ineffective by a host immune response (*i.e.*, desensitization to the treatment and/or dose level is significantly

decreased), and the individual receiving the treatment is less likely to suffer adverse side effects as a result of such an immune response.

Results of experiments demonstrating these and related findings are presented below.

5

A. Orally-Administered IFN γ Inhibits Development of EAE

In experiments detailed in Example 6, orally-administered and injected IFN- γ was tested for its ability to prevent the induction of EAE. EAE was induced in New Zealand White (NZW) mice by immunization with bovine myelin basic protein (bMBP). Recipient
10 NZW mice received OvIFN γ by either i.p. injection or oral feeding 48 hours prior to, on the day of, and 48 hours after immunization with bovine myelin basic protein (bMBP) for induction of experimental allergic encephalomyelitis (EAE).

Both oral feeding and i.p. injection of OvIFN γ protected against EAE (Example 6, Table 6). All animals that received IFN γ via i.p. injection, and 7 of 9 animals that received
15 IFN γ orally, were protected from symptoms of EAE. Furthermore, anti-OvIFN γ monoclonal antibody HL127 was effective at partially neutralizing the ability of the OvIFN γ to block EAE. These experiments demonstrate that orally-administered IFN γ is effective in treating symptoms of EAE, an animal model of multiple sclerosis.

20

B. OvIFN γ is Present in Sera Following Oral Administration.

To confirm that orally-administered IFN γ enters the circulation, the sera of mice that received IFN γ by i.p injection or by oral administration were tested for the presence of IFN γ using a cytopathic effect (antiviral) assay (Familetti, *et al.*, 1981) as described in Example 7.

25

The results are shown in Fig. 6. Specific activities are expressed in antiviral units/mg protein obtained from antiviral assays using MDBK cells. OvIFN γ was detected for up to two 2 hours following oral feeding (filled bars) at levels of 200 U/ml. These data indicate that orally-administered IFN γ enters the circulation and remains in serum for about two hours after being administered.

30

C. OvIFN γ Prevents Chronic Relapse of EAE

In addition to preventing the onset of symptoms associated with EAE, orally-administered OvIFN γ prevents paralysis in a chronic-relapsing model of EAE, as detailed in Example 8. Whereas 5/5 mice immunized with MBP (to induce EAE) which did not

receive OvIFN τ treatment developed chronic relapsing paralysis, 4/5 animals treated with OvIFN τ (either i.p. injection or oral feeding, administered every 48 hours) were fully protected from the disease (Figs. 7B and 7C). These data further support the results described above, and indicate that oral administration of IFN τ can block the development of chronic relapsing EAE. The experiments also suggest that orally-administration of IFN τ as infrequently as once every 48 hours, over an extended period of time, is as effective as i.p. injection at treating a disease condition responsive to treatment by interferon-tau.

10 D. Histological Analyses of Spinal Chord from EAE Mice following Oral Administration of IFN τ .

The ability of OvIFN τ to prevent EAE was also assayed by analyzing the effect of OvIFN τ treatment on cellular consequences of the disease, manifested in the central nervous system (CNS) as lymphocytic lesions in spinal cord white matter. The lesions are indicative of the extent of lymphocyte infiltration into the CNS. MBP-immunized mice were either
15 not treated (control) or treated with OvIFN τ by oral or i.p. routes, and sections of the spinal cord lumbar region were stained and evaluated for lymphocytes as described in Example 9. Lymphocytic lesions were present in spinal cord white matter of control animals (Fig. 8A), but not in mice treated with OvIFN τ by i.p. injection (Fig. 8B) or oral feeding (Fig. 8C). These data indicate that the protective effect of IFN τ is associated with
20 inhibition of lymphocyte infiltration of the CNS. Further, the data demonstrate that IFN τ treatment inhibits cellular manifestation of the autoimmune disease, rather than simply masking symptoms.

25 E. Cessation of Treatment with OvIFN τ Results in Relapsing Paralysis.

Experiments detailed in Example 11 were performed to determine the type and duration of treatment effective to prevent EAE in mice injected with MBP. The mice were protected from EAE by OvIFN τ treatment via i.p. injection or oral feeding (every 48 hours) as long as the treatment persisted (58 days in Example 11), but developed symptoms of the disease after OvIFN τ treatment was stopped (Figure 10). These results suggest that while
30 IFN τ may not cure an autoimmune condition like EAE (e.g., MS), it is an effective treatment that inhibits the pathological manifestations of the condition so long as treatment is continued.

F. Oral Administration of OvIFN γ Reduces Anti-OvIFN γ Antibody Response.

As detailed in Example 12, one advantage of orally-administered (as opposed to injected) IFN γ treatment is a reduction in the anti-IFN γ antibody titer in individuals receiving the oral treatment. After removal of OvIFN γ treatment, mice from each treatment group were bled and sera were examined for the presence of anti-OvIFN γ antibodies by ELISA. Whereas mice receiving IFN γ by i.p. injection exhibited elevated levels of anti-IFN γ antibodies, animals receiving IFN γ by oral feeding exhibited much lower anti-IFN γ antibody titers (typically 3 to 5 -fold lower). As expected mice which received no OvIFN γ treatment displayed no anti-OvIFN γ antibodies.

The sera were also examined for their ability to neutralize OvIFN γ antiviral activity on the MDBK cell line. None of the sera from either i.p. injected or orally fed mice possessed neutralizing activity (Table 7). These results suggest that oral feeding of OvIFN γ largely circumvents an antibody response directed against the OvIFN γ protein. Such a reduced antibody response in orally-treated subjects reduces the chance of undesirable immune system-related side effects of IFN γ treatment.

VI. Applications

A. IFN γ as a Treatment for Immune System Disorders

Diseases which may be treated using methods of the present invention include autoimmune, inflammatory, proliferative and hyperproliferative diseases, as well as cutaneous manifestations of immunologically mediated diseases. In particular, methods of the present invention are advantageous for treating conditions relating to immune system hypersensitivity. There are four types of immune system hypersensitivity (Clayman). Type I, or immediate/anaphylactic hypersensitivity, is due to mast cell degranulation in response to an allergen (*e.g.*, pollen), and includes asthma, allergic rhinitis (hay fever), urticaria (hives), anaphylactic shock, and other illnesses of an allergic nature. Type II, or autoimmune hypersensitivity, is due to antibodies that are directed against perceived "antigens" on the body's own cells. Type III hypersensitivity is due to the formation of antigen/antibody immune complexes which lodge in various tissues and activate further immune responses, and is responsible for conditions such as serum sickness, allergic alveolitis, and the large swellings that sometimes form after booster vaccinations. Type IV hypersensitivity is due to the release of lymphokines from sensitized T-cells, which results in an inflammatory reaction. Examples include contact dermatitis, the rash of measles, and "allergic" reactions to certain drugs.

The mechanisms by which certain conditions may result in hypersensitivity in some individuals are generally not well understood, but may involve both genetic and extrinsic factors. For example, bacteria, viruses or drugs may play a role in triggering an autoimmune response in an individual who already has a genetic predisposition to the autoimmune disorder. It has been suggested that the incidence of some types of hypersensitivity may be correlated with others. For example, it has been proposed that individuals with certain common allergies are more susceptible to autoimmune disorders.

Autoimmune disorders may be loosely grouped into those primarily restricted to specific organs or tissues and those that affect the entire body. Examples of organ-specific disorders (with the organ affected) include multiple sclerosis (myelin coating on nerve processes), type I diabetes mellitus (pancreas), Hashimoto's thyroiditis (thyroid gland), pernicious anemia (stomach), Addison's disease (adrenal glands), myasthenia gravis (acetylcholine receptors at neuromuscular junction), rheumatoid arthritis (joint lining), uveitis (eye), psoriasis (skin), Guillain-Barré Syndrome (nerve cells) and Grave's disease (thyroid). Systemic autoimmune diseases include systemic lupus erythematosus and dermatomyositis.

Other examples of hypersensitivity disorders include asthma, eczema, atopic dermatitis, contact dermatitis, other eczematous dermatitides, seborrheic dermatitis, rhinitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia areata, atherosclerosis, primary biliary cirrhosis and nephrotic syndrome. Related diseases include intestinal inflammations, such as Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, inflammatory bowel disease, Crohn's disease and ulcerative colitis, as well as food-related allergies.

Autoimmune diseases particularly amenable for treatment using the methods of the present invention include multiple sclerosis, type I (insulin dependent) diabetes mellitus, lupus erythematosus, amyotrophic lateral sclerosis, Crohn's disease, rheumatoid arthritis, stomatitis, asthma, uveitis, allergies and psoriasis.

Medicaments containing IFN γ may be used to therapeutically treat and thereby alleviate symptoms of autoimmune disorders such as those discussed above. Treatments with such medicaments are exemplified herein with respect to the treatment of EAE, an animal model for multiple sclerosis.

B. IFN τ as Treatment for Reproductive Disorders.

Although IFN τ bears some similarity to the IFN α family based on structure and its potent antiviral properties, the IFN α s do not possess the reproductive properties associated with IFN τ . For example, recombinant human IFN α had no effect on interestrus interval
5 compared to IFN τ , even when administered at twice the dose (Davis, *et al.*, 1992).

Therefore, although IFN τ has some structural similarities to other interferons, it has very distinctive properties of its own: for example, the capability of significantly influencing the biochemical events of the estrous cycle.

The IFN τ compositions of the present invention can be used in methods of enhancing fertility and prolonging the life span of the *corpus luteum* in female mammals as
10 generally described in Hansen, *et al.* (1991). According to the teachings herein, such methods of enhancing fertility include oral administration of a medicament containing IFN τ in a therapeutically or pharmaceutically effective amount. Further, the compositions may be similarly employed to regulate growth and development of uterine and/or fetal-placental
15 tissues. Compositions containing human IFN τ are particularly useful for treatment of humans, since potential antigenic responses are less likely using a same-species protein.

C. IFN τ as an Antiviral Treatment

The antiviral activity of IFN τ has broad therapeutic applications without the toxic
20 effects that are usually associated with IFN α s. As described above, IFN τ exerts its therapeutic activity without adverse effects on the cells. The relative lack of cytotoxicity of IFN τ makes it extremely valuable as an *in vivo* therapeutic agent and sets IFN τ apart from most other known antiviral agents and all other known interferons.

Formulations or medicaments containing IFN τ can be orally-administered to inhibit
25 viral replication. Further, the compositions can be employed in methods for affecting the immune relationship between fetus and mother, for example, in preventing transmission of maternal viruses (*e.g.*, HIV) to the developing fetus. Compositions containing a human interferon- τ are particularly useful for treatment of humans, since potential antigenic responses are less likely using a homologous protein.

30 Examples of specific viral diseases which may be treated by orally-administered IFN τ include, but are not limited to, hepatitis A, hepatitis B, hepatitis C, non-A, non-B, non-C hepatitis, Epstein-Barr viral infection, HIV infection, herpes virus (EB, CML, herpes simplex), papilloma, poxvirus, picorna virus, adeno virus, rhino virus, HTLV I, HTLV II, and human rotavirus.

D. IFN γ as an Antiproliferative Treatment

IFN γ exhibits potent anticellular proliferation activity. Accordingly, pharmaceutical compositions or medicaments containing IFN γ suitable for oral administration can be used to inhibit cellular growth without the negative side effects associated with other interferons which are currently known. Such compositions or formulations can be used to inhibit, prevent, or slow tumor growth.

Examples of specific cell proliferation disorders which may be treated by orally-administered IFN γ include, but are not limited to, hairy cell leukemia, Kaposi's Sarcoma, chronic myelogenous leukemia, multiple myeloma, superficial bladder cancer, skin cancer (basal cell carcinoma and malignant melanoma), renal cell carcinoma, ovarian cancer, low grade lymphocytic and cutaneous T cell lymphoma, and glioma.

Furthermore, the development of certain tumors is mediated by estrogen. Experiments performed in support of the present invention indicate that IFN γ can suppress estrogen receptor numbers. Therefore, the IFN γ -containing compositions may be particularly useful in the treatment or prevention of estrogen-dependent tumors.

E. Veterinary Applications

In addition to the uses of the methods of the present invention detailed above, it will be appreciated that the methods may be applied to the treatment of a variety of immune system disorders suffered by domesticated and wild animals. For example, hypothyroidism in dogs typically results from a progressive destruction of the thyroid, which may be associated with Lymphocytic thyroiditis (Kempainen and Clark, 1994). Lymphocytic thyroiditis, which resembles Hashimoto's thyroiditis in humans, is thought to be an autoimmune disorder. According to the guidance presented herein, hypothyroidism due to Lymphocytic thyroiditis in dogs may be treated with medicaments containing IFN γ as described above.

Another type of autoimmune disorder in dogs that may be alleviated by treatment with IFN γ is characterized by antinuclear antibody (ANA) positivity, pyrexia and seronegative arthritis (Day, *et al.*, 1985). Immune-mediated thrombocytopenia (ITP; Kristensen, *et al.*, 1994; Werner, *et al.*, 1985), systemic lupus erythematosus (Kristensen, *et al.*, 1994), and leukopenia and Coomb's positive hemolytic anemia (Werner, *et al.*, 1985), may also be amenable to treatment using methods and compositions of the present invention.

VII. Administration of IFN γ A. Pharmaceutical Compositions.

Therapeutic preparations or medicaments containing IFN γ or related polypeptides or proteins can be formulated and manufactured according to known methods for preparing
5 pharmaceutically useful compositions (medicaments). Formulations comprising interferons or interferon-like compounds have been previously described (*e.g.*, Martin, 1976). In general, the IFN γ -containing medicaments will be formulated such that an effective amount
10 of the IFN γ is combined with a suitable carrier and/or excipient in order to facilitate effective administration of the composition. IFN γ , or related polypeptides, may be adminis-
tered to a patient in any pharmaceutically acceptable dosage form, including intravenous, intramuscular, intralesional, or subcutaneous injection. Specifically, compositions and
methods used for other interferon compounds can be used for the delivery of these com-
pounds.

In the case of compositions suitable for oral administration, tablets and capsules
15 containing IFN γ may be manufactured from IFN γ (*e.g.*, lyophilized IFN γ protein) and, optionally, additives such as pharmaceutically acceptable carriers (*e.g.*, lactose, corn starch, light silicic anhydride, microcrystalline cellulose, sucrose), binders (*e.g.*, alpha-form starch, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethyl-
cellulose, polyvinylpyrrolidone), disintegrating agents (*e.g.*, carboxymethylcellulose
20 calcium, starch, low substituted hydroxy-propylcellulose), surfactants (*e.g.*, Tween 80, polyoxyethylene-polyoxypropylene copolymer), antioxidants (*e.g.*, L-cysteine, sodium sulfite, sodium ascorbate), lubricants (*e.g.*, magnesium stearate, talc), and the like.

Further, IFN γ polypeptides can be mixed with a solid, pulverulent or other carrier, for example lactose, saccharose, sorbitol, mannitol, starch, such as potato starch, corn
25 starch, millopectine, cellulose derivative or gelatine, and may also include lubricants, such as magnesium or calcium stearate, or polyethylene glycol waxes compressed to the formation of tablets. By using several layers of the carrier or diluent, tablets operating with slow release can be prepared.

Liquid preparations for oral administration can be made in the form of elixirs,
30 syrups or suspensions, for example solutions containing from about 0.1% to about 30% by weight of IFN γ , sugar and a mixture of ethanol, water, glycerol, propylene glycol and possibly other additives of a conventional nature.

B. Dosage

An orally-active IFN γ pharmaceutical composition is administered in a therapeutically-effective amount to an individual in need of treatment. The dose may vary considerably and is dependent on factors such as the seriousness of the disorder, the age and the weight of the patient, other medications that the patient may be taking and the like. This amount or dosage is typically determined by the attending physician. The dosage will typically be between about 1×10^5 and 1×10^8 units/day, preferably between about 1×10^6 and 1×10^7 units/day. It will be appreciated that because of its lower toxicity, IFN γ can be administered at higher doses than, for example, IFN β . By way of comparison, patients with multiple sclerosis (MS) were treated with 10^6 U and 8×10^6 U of IFN β . Patients receiving 8×10^6 U suffered fewer relapses of disease than did patients receiving 10^6 U. However, patients receiving the higher dose of IFN β (8×10^6 U) also exhibited more side-effects associated with IFN β 's toxicity (IFN β Multiple Sclerosis Study Group). In view of the lower toxicity of IFN γ , these higher effective dosages could be administered without the associated toxic side-effects.

Disorders requiring a steady elevated level of IFN γ in plasma will benefit from oral administration as often as about every two to four hours or administration via injection about every 12-24 hours, while other disorders, such as MS, may be effectively treated by administering a therapeutically-effective dose at less frequent intervals, e.g., once every 48 hours. The rate of administration of individual doses is typically adjusted by an attending physician to enable administration of the lowest total dosage while alleviating the severity of the disease being treated.

Once improvement of a patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained.

Autoimmune disorders effecting the skin, such as psoriasis, can be treated intra-lesionally using IFN γ , wherein formulation and dose will depend on the method of administration and on the size and severity of the lesion to be treated. Preferred methods include intradermal and subcutaneous injection. Multiple injections into large lesions may be possible, and several lesions on the skin of a single patient may be treated at one time. The schedule for administration can be determined by a person skilled in the art. Formulations designed for sustained release can reduce the frequency of administration.

Regional treatment with the IFN γ polypeptides of the present invention is useful for treatment of autoimmune diseases in specific organs. Treatment can be accomplished by intraarterial infusion or intravenous injection. A catheter can be surgically or angiographically implanted to direct treatment to the affected organ. A subcutaneous portal, connected to the catheter, can be used for chronic treatment, or an implantable, refillable pump may also be employed.

Alternatively, the composition may be administered by direct injection into the affected tissue. For treating rheumatoid arthritis, for example, the composition may be administered by direct injection into the affected joint. The patient may be treated at repeated intervals of at least 24 hours, over a several week period following the onset of symptoms of the disease in the patient.

Systemic treatment is essentially equivalent for all applications. Systemic treatment using oral administration is discussed above. Multiple intravenous or subcutaneous doses are also possible, and in the case of implantable methods for treatment, formulations designed for sustained release are particularly useful. Patients may also be treated using implantable subcutaneous portals, reservoirs, or pumps. Other methods of administration include suppository and intra-vaginal. For the treatment of systemic lupus erythematosus (SLE) or MS, for example, the composition may be administered by oral or parenteral administration, such as IV administration.

20

C. Combination Therapies

It will, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies. For example, in view of IFN γ 's relative lack of toxicity at high dosages, MS patients that do not show improvement at IFN β 1b's low dosage or could not tolerate IFN β 1b due to toxicity may benefit from subsequent or simultaneous treatment with higher dosages of IFN γ or peptides derived therefrom. In this respect, IFN β 1b may be considered a "second treatment agent". Further, development of neutralizing antibodies has been demonstrated in IFN β 1b treated patients (Weinstock-Guttman, *et al.*, 1995). In cases where such neutralizing antibodies prove to impede the effectiveness of IFN β 1b, IFN γ may be an important alternative therapy, since antibody cross-reactivity is unlikely to occur, and IFN γ is unlikely to generate neutralizing antibodies (see Example 12). Orally-administered IFN γ is particularly advantageous in this respect, since it causes a significantly lower anti-IFN γ antibody response than injected IFN γ .

Another type of combination therapy enabled by the present invention is the oral administration of an antigen against which an autoimmune response is directed in combination with IFN γ . Oral administration of such an antigen can result in tolerization, reducing the severity of the autoimmune disease (for review, see, *e.g.*, Weiner, *et al.*, 1994). It is contemplated that the IFN γ has a synergistic effect with the tolerization induced by the antigen, thereby alleviating the severity of the autoimmune disease. For example, MBP has been shown to suppress EAE (Lider, *et al.*, 1989). According to the methods of the present invention, MBP may be administered (as a "second treatment agent") in combination with IFN γ to treat multiple sclerosis. Other examples include administration of IFN γ with collagen to treat rheumatoid arthritis, and with acetylcholine receptor polypeptides to treat myasthenia gravis.

Furthermore, IFN γ may be orally administered with known immunosuppressants, *e.g.*, steroids, to treat autoimmune diseases such as multiple sclerosis. The immunosuppressants (considered "second treatment agents") may act synergistically with IFN γ and result in a more effective treatment that could be obtained with an equivalent dose of IFN γ or the immunosuppressant alone.

Similarly, in a treatment for a cancer or viral disease, IFN γ may be administered in conjunction with, *e.g.*, a therapeutically effective amount of one or more chemotherapy agents such as busulfan, 5-fluoro-uracil (5-FU), zidovudine (AZT), leucovorin, melphalan, prednisone, cyclophosphamide, dacarbazine, cisplatin, and dipyridamole.

The following examples illustrate but in no way are intended to limit the present invention.

MATERIALS AND METHODS

A. Buffers

Phosphate-buffered saline (PBS)

10 \times stock solution, 1 liter:

80 g NaCl

2 g KCl

11.5 g Na₂HPO₄-7H₂O

2 g KH₂PO₄

Working solution, pH 7.3:

137 mM NaCl

2.7 mM KCl

4.3 mM Na₂HPO₄-7H₂O

1.4 mM KH₂PO₄

B. General ELISA Protocol for Detection of Antibodies

Polystyrene 96 well plates Immulon II (PGC) were coated with 5 $\mu\text{g/mL}$ (100 μL per well) antigen in 0.1 M carbonate/bicarbonate buffer, pH 9.5. The plates were sealed with parafilm and stored at 4°C overnight.

- 5 Following incubation, the plates were aspirated and blocked with 300 μL 10% NGS and incubated at 37°C for 1 hr. The plates were then washed 5 times with PBS 0.5% "TWEEN-20". Antisera were diluted in 0.1 M PBS, pH 7.2. The desired dilution(s) of antisera (0.1 mL) were added to each well and the plates incubated 1 hour at 37°C. The plates were then washed 5 times with PBS 0.5% "TWEEN-20".

- 10 Horseradish peroxidase (HRP) conjugated goat anti-human antiserum (Cappel, Durham, NC) was diluted 1/5,000 in PBS. 0.1 mL of this solution was added to each well. The plate was incubated 30 min at 37°C, then washed 5 times with PBS.

- Sigma ABTS (substrate) was prepared just prior to addition to the plate. The reagent consists of 50 mL 0.05 M citric acid, pH 4.2, 0.078 mL 30% hydrogen peroxide solution and 15 mg ABTS. 0.1 mL of the substrate was added to each well, then incubated for 30 min at room temperature. The reaction was stopped with the addition of 0.050 mL 5% SDS (w/v). The relative absorbance is determined at 410 nm.

C. Production of OvIFN- γ

- 20 A synthetic OvIFN γ gene was generated using standard molecular methods (Ausubel, *et al.*, 1988) by ligating oligonucleotides containing contiguous portions of a DNA sequence encoding the OvIFN γ amino acid sequence (Imakawa, *et al.*, 1987). The resulting IFN γ polynucleotide coding sequence spans position 16 through 531: a coding sequence of 172 amino acids.

- 25 The full length synthetic gene *StuI/SstI* fragment (540 bp) was cloned into a modified pIN III omp-A expression vector and transformed into a competent SB221 strain of *E. coli*. For expression of the IFN γ protein, cells carrying the expression vector were grown in L-broth containing ampicillin to an OD (550 nm) of 0.1-1, induced with IPTG (isopropyl-1-thio-b-D-galactoside) for 3 hours and harvested by centrifugation. Soluble recombinant IFN γ was liberated from the cells by sonication or osmotic fractionation.

30 For expression in yeast, the IFN γ gene was amplified using polymerase chain reaction (PCR: Mullis, 1987; Mullis, *et al.*, 1987) with PCR primers containing *StuI* and *SacI* restriction sites at the 5' and 3' ends, respectively. The amplified fragments were digested with *StuI* and *SacII* and ligated into the *SacII* and *SmaI* sites of

"pBLUESCRIPT+(KS)", generating pBSY-IFN τ . Plasmid pBSY-IFN τ was digested with *Sac*II and *Eco*RV and the fragment containing the synthetic IFN τ gene was isolated. The yeast expression vector pBS24Ub (Sabin, *et al.*, 1989; Ecker, *et al.*, 1989) was digested with *Sal*I. Blunt ends were generated using T4 DNA polymerase. The vector DNA was
5 extracted with phenol and ethanol precipitated (Sambrook, *et al.*, 1989). The recovered plasmid was digested with *Sac*II, purified by agarose gel electrophoresis, and ligated to the *Sac*II-*Eco*RV fragment isolated from pBSY-IFN τ . The resulting recombinant plasmid was designated pBS24Ub-IFN τ .

The recombinant plasmid pBS24Ub-IFN τ was transformed into *E. coli*. Recombi-
10 nant clones containing the IFN τ insert were isolated and identified by restriction enzyme analysis. IFN τ coding sequences were isolated from pBS24Ub-IFN τ and cloned into a *Pichia pastoris* vector containing the alcohol oxidase (AOX1) promoter (Invitrogen, San Diego, CA). The vector was then used to transform *Pichia pastoris* GS115 His^r host cells and protein was expressed following the manufacturer's instructions. The protein was
15 secreted into the medium and purified by successive DEAE-cellulose and hydroxyapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining. The purified protein had a specific activity of about 0.29 to about 0.44×10^8 U/mg as measured by anti-viral activity on Madin-Darby bovine kidney (MDBK) cells.

20

EXAMPLE 1

Toxicity of IFN β , IFN γ and IFN τ

A. In Vivo Toxicity - Cell Counts and Weight Changes

The effects of *in vivo* treatment with IFN τ , IFN β and IFN α (10^5 U/injection) on total white blood cell (WBC), total lymphocyte counts and weight measurements in NZW
25 mice were assessed as follows. Interferons (OvIFN τ , MuIFN β , and MuIFN α) were injected intraperitoneally (i.p.) at a concentration of 10^5 U in a total volume of 0.2 ml in PBS into groups of New Zealand White (NZW) mice (Jackson Laboratories, Bar Harbor, ME). Three to four animals were included in each group. White blood cell (WBC) counts were determined before injection and at selected timepoints thereafter (typically 12 and 24
30 hours) using a hemocytometer and standard techniques. Differential WBC counts were performed on Wright-Giemsa stained blood smears. Before injection, the weights of the animals ranged from 20 to 23 grams. The results are summarized in Table 3, below.

TABLE 3

IN VIVO TOXICITY OF INTERFERONS AS MEASURED
BY WHITE BLOOD CELL COUNTS AND PERCENT WEIGHT CHANGE

| IFN | Cell Count (Cell No. $\times 10^3$) | | | | % Lymphocyte Depression | % Weight Change 24 Hours after Injection |
|----------|--------------------------------------|---------------|------------------------|---------------|-------------------------|--|
| | Before Injection | | 12 hr. after Injection | | | |
| | Total WBC | Lymphocytes | Total WBC | Lymphocytes | | |
| none | 7.3 \pm 1.0 | 6.4 \pm 0.7 | 8.0 \pm 0.8 | 7.1 \pm 0.7 | 0 | +0.5 \pm 0.7 |
| τ | 6.7 \pm 0.7 | 5.9 \pm 0.6 | 6.7 \pm 0.5 | 5.8 \pm 0.4 | 1.7 | +1.3 \pm 0.5 |
| β | 7.0 \pm 1.4 | 6.0 \pm 0.5 | 6.8 \pm 0.8 | 4.1 \pm 0.3 | 31.7 | -20.0 \pm 1.0 |
| α | 6.0 \pm 0.8 | 5.2 \pm 0.7 | 4.8 \pm 0.5 | 2.3 \pm 0.2 | 55.8 | -8.5 \pm 2.0 |

No significant differences in WBC counts, lymphocyte counts or weight change were observed between IFN τ -treated and untreated mice. In contrast, IFN β -treated mice exhibited a 31.7% depression in lymphocyte counts 12 hours after injection, which continued for at least the next 12 hours. IFN α -treated mice exhibited a 55.8% lymphocyte depression and significant weight loss 12 hours after injection. These data indicate that, unlike IFN β and IFN α , IFN τ lacks toxicity *in vivo* at the above concentrations as evidenced by peripheral blood cell counts and weight measurements.

B. In Vitro Toxicity - L929 Cell Assay

The toxicity of IFN treatment was measured *in vitro* using the mouse L929 cell line. L929 cells were treated with 6000 U/ml to 200,000 U/ml of either OvIFN τ or MuIFN β . The interferons were added at time zero and the cells were incubated for 72 hours and stained with crystal violet. The percentage of living cells was determined by measuring the absorbance at 405 nm.

Exemplary data are shown in Figure 1. Values are presented as percent viability \pm standard error in which 100 percent is equal to the viability of L929 cells treated with media alone. At 6000 U/ml, IFN β -treated cells exhibited a 77.0 \pm 0.6% viability. Viability of L929 cells decreased as the concentrations of IFN β increased in a dose-dependent manner. In contrast, L929 cells showed no decrease in viability at any of the IFN τ concentrations tested. These data indicate that, unlike IFN β , IFN τ lacks toxicity at high concentrations *in vitro*.

Taken together, the results summarized above demonstrate that IFN τ is essentially non-toxic at concentrations at which IFN β induces toxicity both *in vitro* and *in vivo*.

EXAMPLE 2IFN τ Inhibits Development of Experimental
Allergic Encephalomyelitis

IFN- τ was tested for its ability to prevent the induction of EAE. Recipient NZW mice were injected i.p. with either a single dose of 10^5 U/ml recombinant ovine IFN-tau (OvIFN τ) or murine IFN-beta (MuIFN- β ; Lee Biomolecular, San Diego, CA) on the day of immunization with bovine myelin basic protein (bMBP) for induction of EAE or 3 doses of 10^5 U/ml of OvIFN- τ or MuIFN- β 48 hours before, on the day of and 48 hours after immunization with MBP for induction of EAE.

For induction of EAE, 300 μ g of bMBP was emulsified in complete Freund's adjuvant containing 8 mg/ml of H37Ra and injected on either side of the base of the tail. On the day of immunization and 48 hours later, 400 ng of Pertussis toxin (List Biologicals, Campbell, CA) was also injected. Mice were examined daily for signs of EAE and severity of disease was graded on the following scale: 1, loss of tail tone; 2, hind limb weakness; 3, paraparesis; 4, paraplegia; 5, moribund/death.

Table 4Effects of IFN- τ on Development of EAE

| Treatment | # of IFN Doses | Disease Incidence | Mean Day of Onset | Mean Severity |
|----------------|----------------|-------------------|-------------------|---------------|
| none | 0 | 5/5 | 16.2 \pm 0.8 | 3.0 \pm 1.0 |
| oIFN τ | 1 | 5/5 | 23.8 \pm 0.5 | 2.0 \pm 1.0 |
| MuIFN- β | 1 | 4/4 | 23.5 \pm 0.6 | 2.1 \pm 1.6 |
| None | 0 | 7/9 | 15.3 \pm 1.4 | 2.6 \pm 0.8 |
| oIFN τ | 3 | 0/9 | -- | -- |
| MuIFN- β | 3 | 1/9 | 22 | 0.5 |

The results of the experiments are summarized in Table 4, above. The data are split into two sets. The first set (first three rows) corresponds to experiments where IFN was injected into experimental animals on the day of the immunization. All of the animals in this set developed EAE, but the mean day of onset was delayed in both the OvIFN τ (23.8 \pm 0.5 days) and MuIFN- β (23.5 \pm 0.6 days) treated animals relative to control animals (16.2 \pm 0.8 days). Further, the mean severity of the disease, quantitated as

described above, was reduced in both IFN-treated groups relative to controls. Like OvIFN γ , a single dose of 10^5 U of MuIFN β also caused a 7 day delay in the development of disease.

The results are more striking for the multiple dose protocol (rows 4-6 of Table 1), where three doses of IFN (48 hours prior, day of, and 48 hours post immunization) were administered to the experimental animals. Although seven of the nine control animals developed EAE an average of 15.2 days following immunization, none of nine animals treated with OvIFN γ developed the disease. Of the nine animals treated with MuIFN- β , one succumbed to EAE 22 days after immunization.

A time course of the mean severity from the experiments described above is presented in Figure 2. Data from control animals are indicated by (Δ), data from animals treated with a single dose of OvIFN γ are indicated by (\oplus), and data from animals that received 3 doses of OvIFN γ are indicated by (\square).

The data demonstrate that IFN γ is an effective immunotherapy for the prevention of EAE and is as effective a treatment as MuIFN β in this model of autoimmune disease. Taken together with the lower toxicity of IFN γ relative to IFN β , the data suggest that treatment of individuals having an autoimmune disorder (such as multiple sclerosis) with IFN γ may be preferable and more effective than treatment with IFN β .

EXAMPLE 3

IFN γ Inhibition of T-Cell Proliferation

The effects of IFN γ on proliferation of spleen cells from MBP-immunized NZW mice stimulated with MBP *in vitro* were determined as follows. Spleen cells from NZW mice immunized with bMBP were cultured in 300 μ g/ml of bMBP in the presence of [3 H]thymidine and 0, 10, 100, or 1000 U/ml of OvIFN γ . Proliferation was measured by [3 H]thymidine incorporation.

The results are shown in Figure 3. Data are presented as mean counts per minute (cpm) of triplicate samples. Background cpm have been subtracted from the cpm values presented. Proliferation in response to MBP was vigorous and could be reduced by IFN γ in a dose-dependent manner. 1000 U/ml IFN γ reduced proliferation to less than half of that observed in response to MBP alone.

These results demonstrate that IFN γ has antiproliferative activity against T cells specific for the autoantigen, MBP, and are consistent with the observation that IFN γ inhibits or eliminates symptoms of MBP-induced EAE.

EXAMPLE 4IFN γ Prevents Superantigen Reactivation

IFN γ was examined for its ability to prevent superantigen reactivation of EAE in NZW mice (Jackson Laboratory, Bar Harbor, ME). Schematic diagrams of the protocol followed in these experiments are shown in Figures 4A, 4B, 4C, 4D, 4E and 4F. These figures are referred to in the context of the protocol described below.

For induction of EAE, 300 μ g of bMBP and 400 ng of Pertussis toxin (List Biological Technologies, Campbell, CA) were emulsified in complete Freund's adjuvant containing 8 mg/ml of H37Re and injected on either side of the base of the tail (Fig. 4A). Another injection containing 400 ng of Pertussis toxin was administered 48 hours later. The injections induced EAE, which peaked (Fig. 4B) and gradually tapered off, such that eventually, all clinical symptoms of EAE were resolved (Fig. 4C).

SEB was administered one month after resolution of disease (Fig. 4D). The mice were injected i.p. with 3 doses of 10^5 U of IFN γ 48 hours before, on the day of, and 48 hours after injection of 40 μ g SEB (Toxin Technology, Sarasota, FL) and 400 ng of Pertussis toxin (List Biological Technologies, Campbell, CA) (in 0.2 ml PBS) for superantigen reactivation. Control mice received SEB and Pertussis toxin only. The IFN γ preparation was identical to that described in Example 2. Mice were examined daily for signs of EAE and severity of disease was graded as described in Example 2.

TABLE 5IFN γ PREVENTS SUPERANTIGEN REACTIVATION OF EAE

| Treatment | No. of IFN Doses | Disease Incidence | Mean Day of Onset | Mean Severity |
|----------------|------------------|-------------------|-------------------|---------------|
| <u>Expt. 1</u> | | | | |
| none | 0 | 3/4 | 6.0 ± 1.7 | 1.6 ± 0.5 |
| OvIFN γ | 3 | 0/4 | -- | -- |
| <u>Expt. 2</u> | | | | |
| none | 0 | 3/5 | 10.0 ± 2.5 | 1.4 ± 0.4 |
| OvIFN γ | 3 | 0/5 | -- | -- |

The data are summarized in Table 5, above. Of a total of nine control mice (receiving no IFN γ), six developed a reactivation of EAE. The mean day of onset was 6 ± 1.7 in the first experiment, and 10 ± 2.5 in the second experiment. Mean severity of the

disease was 1.6 ± 0.5 in the first experiment, and 1.4 ± 0.4 in the second experiment. Of the nine animals that were treated with $\text{IFN}\gamma$, however, none developed symptoms of the disease.

5

EXAMPLE 5

$\text{IFN}\gamma$ Inhibits $\text{V}\beta$ -specific T-Cell Activation

The effect of $\text{IFN}\gamma$ treatment of SEB-induced $\text{V}\beta$ specific T cell expansion *in vitro*

was evaluated as follows. FACS reagents were obtained from Pharmingen, San Diego, CA. $\text{V}\beta$ specific T-cell FACS analysis was performed on naive, SEB-injected (50 μg) or $\text{IFN}\gamma$ (10⁵ U) and SEB (50 μg) injected NZW mice. All injections were i.p. and were administered as described in Example 3. Analyses were performed 72 hours after the injections.

For FACS analysis, $\sim 10^6$ T cells were isolated from the animals and incubated with biotin-labeled anti- $\text{V}\beta$ antibodies for 45 minutes. The cells were then washed and incubated with streptavidin-phycoerythrin for 15 minutes, followed by another wash and a 45 minute incubation with FITC-labeled anti-CD4 antibodies. The cells were washed again and analyzed on a FACSsort (Becton-Dickinson, Mountain View, CA) in duplicate as 10,000 events per sample.

Results of exemplary experiments are shown in Figure 5. Open bars represent naive animals; closed bars represent SEB-injected animals, and crosshatched bars represent $\text{IFN}\gamma$ - and SEB-injected animals. Values are presented as percentage of positively stained cells \pm standard error. Values for the $\text{V}\beta 8^+ \text{CD}4^+$ T cell subset of SEB-injected and $\text{IFN}\gamma$ - and SEB-injected were significantly different as shown by student's t test ($P < 0.02$). Naive NZW mice exhibited 5.1 ± 0.1 % $\text{V}\beta 8^+ \text{CD}4^+$ T cells. After injection with 50 μg of SEB, this subset was expanded to 10.2 ± 0.2 %. When 10⁵ U of $\text{IFN}\gamma$ preceded SEB injection, expansion of the $\text{V}\beta 8^+ \text{CD}4^+$ T-subset was limited to 7.6 ± 0.2 %. Partial inhibition of $\text{V}\beta 7^+$ and $\text{V}\beta 11^+$ T cells, for which SEB is also specific, was also observed.

These data indicate that treatment with $\text{IFN}\gamma$ can partially inhibit SEB-induced $\text{V}\beta$ T cell expansion *in vivo*, and further support the observation that $\text{IFN}\gamma$ inhibits or eliminates symptoms of MBP-induced EAE.

30

EXAMPLE 6

Orally-Administered Ov $\text{IFN}\gamma$ Blocks Development of Experimental Allergic Encephalomyelitis

Orally-administered and injected $\text{IFN}\gamma$ was tested for its ability to prevent the induction of EAE. Recipient New Zealand White (NZW) mice received Ov $\text{IFN}\gamma$ (10⁵

35

U/ml) by either i.p. injection or oral feeding 48 hours prior to, on the day of, and 48 hours after immunization with bMBP for induction of EAE. 10^5 U of IFN γ were mixed with PBS to a total volume of 100 μ l and administered using a feeding tube placed down the esophagus and into the stomach. The dilution of the IFN γ in PBS was done immediately before administration.

For induction of EAE in NZW mice, 300 μ g of bovine myelin basic protein (bMBP) was emulsified in complete Freund's adjuvant (CFA) containing 8 mg/ml of H37Ra (Mycobacterium tuberculosis, Difco, Detroit, MI) and injected on either side of the base of the tail. On the day of immunization and 48 hours later, 400 ng of Pertussis toxin (List Biologicals, Campbell, CA) was also injected. For induction of EAE in SJL/J mice, the same protocol was used as described except mice were immunized again 7 days after the initial immunization. Mice were examined daily for signs of EAE and severity of disease was graded as described in Example 2.

Anti-OvIFN γ monoclonal antibody (mAb), HL127, was used to determine whether prevention of EAE was specific to OvIFN γ treatment (antibody HL127, directed against aa 139-172 of SEQ ID NO:2, neutralizes the antiviral activity of OvIFN γ in an antiviral assay using the MDBK cell line). A 1:10 dilution of HL127 was incubated for 2 hours with OvIFN γ prior to administration by either i.p. injection or oral feeding. Antibodies directed against IFN γ antigens, may be generated using the information herein combined with known techniques for antibody production (*e.g.*, Harlow, *et al.*, 1988).

The results are shown in Table 6, below. Both oral feeding and i.p. injection of OvIFN γ protected against acute induction of EAE. None of the animals that received IFN γ via i.p. injection developed symptoms of EAE, while of the animals that received IFN γ orally, 7 of 9 (78%) were protected. Antibody HL127 was effective at partially neutralizing the ability of the OvIFN γ to block EAE. These data indicate that orally-administered IFN γ is effective as a treatment in an animal model of multiple sclerosis.

Table 6

Oral Feeding of OvIFN γ Blocks Acute
EAE and Can Be Reversed by an OvIFN γ
Specific Monoclonal Antibody in NZW Mice

| Route of Admini- stration | Treatment | Disease Inci- dence | Mean Day of Onset | Mean Severity |
|------------------------------|------------------------|------------------------|----------------------|------------------|
| i.p. | PBS | 4/4 | 24.8 \pm 2.1 | 2.5 \pm 0 |
| i.p. | OvIFN γ | 0/4 | -- | -- |
| i.p. | OvIFN γ + HL127 | 3/4 | 20.7 \pm 1.2 | 2.3 \pm 0.6 |
| oral | PBS | 7/9 | 22.0 \pm 1.0 | 2.7 \pm 0.6 |
| oral | OvIFN γ | 2/9 | 19 | 3 |
| oral | OvIFN γ + HL127 | 5/8 | 20.7 \pm 0.6 | 3 \pm 0 |

OvIFN γ (10^5 U) was administered 48 hours prior to MBP immunization, on the day of MBP immunization and 48 hours after MBP immunization by either i.p. injection or oral feeding. HL127, a monoclonal antibody specific for OvIFN γ , was incubated with OvIFN γ for two hours prior to administration.

EXAMPLE 7Detection of OvIFN γ in Sera Following Oral Administration

The amount of OvIFN γ detectable in the sera of mice (treated as above) was compared over time after oral feeding (as above) or i.p. injection of OvIFN γ . Mice were administered 3×10^5 U of OvIFN γ and bled at 0.5, 2, 4, 6, 24 and 48 hours following IFN γ administration. Sera were tested in a cytopathic effect (viral plaque) assay (Familetti, *et al.*, 1981) to determine the amount of IFN γ in the samples.

Briefly, dilutions of IFN γ were added to MDBK cells grown to confluency in a flat bottom 96 well plate and incubated for 18 to 24 hours at 37°C. Vesicular stomatitis virus (VSV) was added to the plate for 45 minutes at room temperature. Virus was removed and methyl cellulose was added and the plate incubated for 48 hours at 37°C. After removal of methyl cellulose, the plate was stained with crystal violet for visualization of plaques. For measurement of IFN neutralization, OvIFN γ at a concentration of 500 U/ml was incubated for 1 hour at 37°C with either sera or HL127. One antiviral unit caused a 50% reduction in destruction of the monolayer, relative to untreated MDBK cells

infected with VSV (control plates). All samples were assayed simultaneously to eliminate interassay variability.

As shown in Fig. 6, OvIFN γ was detected at 0.5 hour and 2 hours after oral feeding (filled bars) at levels of 200 U/ml. By comparison, somewhat higher levels of OvIFN γ were detected for over a 24 hour period of time after i.p. injection (open bars). These data show that the above dose of IFN γ can be detected in serum for about two hours following oral administration.

EXAMPLE 8

10 Prevention of Chronic Relapse of Experimental Allergic Encephalomyelitis by Orally-Administered OvIFN γ

The ability of OvIFN γ to prevent paralysis was examined using a chronic-relapsing model of EAE, in which SJL mice immunized with MBP develop a chronic form of the disease where the appearance of symptoms occurs in a relapsing-remitting manner (Zamvil and Steinman, 1990).

EAE was induced in SJL mice essentially as described above. The mice were treated with 10^5 U of OvIFN γ by either i.p. injection or oral feeding on the day of immunization (day 0) and every 48 hours thereafter for the duration of the experiment. As presented in Figure 7A, SJL mice which were immunized with MBP but did not receive OvIFN γ treatment developed chronic relapsing paralysis with a 5/5 incidence of disease, with a peak mean severity of ~ 2.5 occurring 14 days after the start of the experiment. In contrast, treatment with OvIFN γ by either i.p. injection or oral feeding (Figures 7B and 7C, respectively) resulted in protection from EAE. Incidence of disease in both OvIFN γ treatment groups was reduced to 1/5 animals, with a mean severity of ~ 1.0 . These data indicate that oral administration of IFN γ can block the development of chronic relapsing EAE, and suggest that orally-administered IFN γ may be as effective as i.p. injection when the IFN γ is fed about every 48 hours over an extended period of time.

EXAMPLE 9

30 Histological Analysis

Histological analyses were performed to determine the extent of lymphocyte infiltration into the CNS of MBP-immunized mice treated with OvIFN γ by oral and i.p. routes. Mice were perfused with 4% paraformaldehyde, vertebral columns were removed and treated with formalin for 2 to 3 days. Spinal cords were dissected out and soaked in 0.5 % sucrose overnight at 4°C. Spinal cord sections were embedded and sections cut in a

microtome. Sections were fixed to slides in 4 % paraformaldehyde and stained with cresyl violet for visualization of inflammatory infiltrates.

The results are shown in Figures 8A, 8B and 8C at a final magnification of 222 \times . Lymphocytic lesions were present in control spinal cord white matter (Fig. 8A). In contrast, no lymphocytic infiltrates were detected in mice treated with OvIFN γ by i.p. injection (Fig. 8B) or oral feeding (Fig. 8C). These data suggest that the protective effect of IFN γ is associated with inhibition of lymphocyte infiltration of the CNS.

EXAMPLE 10

Induction of IL10 by Treatment with OvIFN γ

During the course of OvIFN γ treatment of SJL for prevention of chronic relapsing EAE, mice were bled and sera were examined for the presence of interleukin 10 (IL10). Sera from mice which received either a single IFN γ (10^5 U) treatment (by i.p. injection or oral feeding), prolonged IFN γ (10^5 U) treatment (by i.p. injection or oral treatment for greater than 20 days) or no treatment were examined for IL10 by enzyme-linked immunosorbent assay (ELISA) using IL10 ELISA kits (Genzyme, Cambridge, MA) following the manufacturer's instructions. All sera samples were tested in duplicate.

No IL10 was detected in control mice or in mice which received a single treatment of OvIFN γ by either i.p. injection or oral feeding. In contrast, SJL mice which received OvIFN γ by either i.p. injection or oral feeding every 48 hours for greater than 20 days had detectable levels of IL10 in their sera (Figure 9). These data suggest that IFN γ -induced production of IL10 may be a contributing mechanism by which OvIFN γ prevents development of EAE.

EXAMPLE 11

Cessation of Treatment with OvIFN γ Results in Relapsing Paralysis

SJL mice which were protected from EAE by OvIFN γ treatment via i.p. injection or oral feeding (every 48 hours) were followed for 58 days, during which time no disease development was observed. Treatment with OvIFN γ was then removed and the mice were observed for an additional 22 days for symptoms of disease.

The results are shown in Figure 10. IFN γ treatment is denoted as plus signs and removal of IFN γ treatment is denoted as minus signs beneath the graph. Disease incidence in each treatment group was as follows: PBS control = 3/4 (square); i.p. injection = 3/3 (triangle); oral feeding = 3/4 (circle).

Both groups of mice which had previously been protected from EAE by OvIFN γ treatment developed signs of paralysis 6 to 12 days after removal of the OvIFN γ treatment. These data indicate that ongoing administration of IFN γ , by either i.p. injection or oral feeding, is desirable for continued protection from EAE in the chronic-relapsing model of

5 EAE.

EXAMPLE 12

Oral Administration of OvIFN γ Reduces Anti-OvIFN γ Antibody Response

After removal of OvIFN γ treatment in the experiments described in Example 11,

10 above, mice from each treatment group were bled and sera were examined for the presence of anti-OvIFN γ antibodies (Ab). The antigen, OvIFN γ , was adsorbed to the flat bottoms of plastic tissue culture wells overnight at a concentration of 600 ng/well, and subsequently evaporated to dryness. The plates were treated with 5% milk (Carnation) in PBS for 2

15 hours in order to block nonspecific binding and then washed 3 times with PBS containing 0.05% Tween 20. Various dilutions of sera from mice which were untreated, OvIFN γ treated by i.p. injection and OvIFN γ treated by oral feeding were added and incubated for 3 hours. Binding was assessed with goat anti-mouse immunoglobulin coupled to horseradish peroxidase. Color development was monitored at 492 nm in an ELISA plate reader (Bio-Rad, Richmond, CA) after o-phenylenediamine and H₂O₂ were added and the reaction

20 terminated with 2M H₂SO₄.

Exemplary results are shown in Figure 11. Sera from untreated, OvIFN γ treated-i.p. injected and OvIFN γ treated-orally fed (2 mice/group) were examined by ELISA using multiple dilutions, including 1:30 (open bars) and 1:120 (filled bars). Mice which received OvIFN γ by oral feeding exhibited minimal Ab levels while mice which received

25 OvIFN γ by i.p. injection exhibited elevated levels of anti-OvIFN γ Ab. As expected, mice which received no OvIFN γ treatment displayed no anti-OvIFN γ Ab.

Sera were also examined for their ability to neutralize OvIFN γ antiviral activity on MDBK cells as described above. The results are shown in Table 7, below. None of the sera from either i.p. injected or orally fed mice possessed neutralizing activity. These data

30 suggest that oral treatment with IFN γ circumvents the Ab response directed against OvIFN γ protein observed in i.p. injection-treated individuals, and that neither treatment typically results in the generation of neutralizing antibodies.

Table 7

Sera from Mice Treated with OvIFN γ by
i.p. Injection or Oral Feeding do
Not Possess Neutralizing Activity

| 500 U/ml of OvIFN γ Cocultured with Sera From: | OvIFN γ Titer (U/ml) |
|--|--------------------------------|
| untreated | 500 |
| i.p. injected | 500 |
| orally fed | 500 |
| HL127 | < 50 |

15 While the invention has been described with reference to specific methods and
embodiments, it is appreciated that various modifications and changes may be made without
departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: University of Florida
- (ii) TITLE OF INVENTION: Method for Treatment of Autoimmune Diseases
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Dehlinger & Associates
 - (B) STREET: 350 Cambridge Ave., Suite 250
 - (C) CITY: Palo Alto
 - (D) STATE: CA
 - (E) COUNTRY: USA
 - (F) ZIP: 94306
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 15-MAR-1996
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/406,190
 - (B) FILING DATE: 16-MAR-1995
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Sholtz, Charles K.
 - (B) REGISTRATION NUMBER: 38,615
 - (C) REFERENCE/DOCKET NUMBER: 5600-0002.41
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 415-324-0880
 - (B) TELEFAX: 415-324-0960

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 516 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 - (A) ORGANISM: Ovis aries
 - (B) STRAIN: Domestic
 - (D) DEVELOPMENTAL STAGE: Blastula (blastocyst)
 - (F) TISSUE TYPE: Trophectoderm
 - (G) CELL TYPE: Mononuclear trophectoderm cells
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: oTP-1a
- (viii) POSITION IN GENOME:

(C) UNITS: bp

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..516

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ott, Troy L
Van Heeke, Gino
Johnson, Howard M
Bazer, Fuller W(B) TITLE: Cloning and Expression in *Saccharomyces cerevisiae* of a Synthetic Gene for the Type I Trophoblast Interferon Ovine Trophoblast Protein-1: Purification and Antiviral Activity

(C) JOURNAL: J. Interferon Res.

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(F) PAGES: 357-364

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(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | | | | | | | | | | | | | | |
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| TGC | TAC | CTG | TCG | CGA | AAA | CTG | ATG | CTG | GAC | GCT | CGA | GAA | AAT | TTA | AAA | 48 |
| Cys | Tyr | Leu | Ser | Arg | Lys | Leu | Met | Leu | Asp | Ala | Arg | Glu | Asn | Leu | Lys | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| CTG | CTG | GAC | CGT | ATG | AAT | CGA | TTG | TCT | CCG | CAC | AGC | TGC | CTG | CAA | GAC | 96 |
| Leu | Leu | Asp | Arg | Met | Asn | Arg | Leu | Ser | Pro | His | Ser | Cys | Leu | Gln | Asp | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| CGG | AAA | GAC | TTC | GGT | CTG | CCG | CAG | GAA | ATG | GTT | GAA | GGT | GAC | CAA | CTG | 144 |
| Arg | Lys | Asp | Phe | Gly | Leu | Pro | Gln | Glu | Met | Val | Glu | Gly | Asp | Gln | Leu | |
| | | 35 | | | | | 40 | | | | | 45 | | | | |
| CAA | AAA | GAC | CAA | GCT | TTC | CCG | GTA | CTG | TAT | GAA | ATG | CTG | CAG | CAG | TCT | 192 |
| Gln | Lys | Asp | Gln | Ala | Phe | Pro | Val | Leu | Tyr | Glu | Met | Leu | Gln | Gln | Ser | |
| | | 50 | | | | 55 | | | | | 60 | | | | | |
| TTC | AAC | CTG | TTC | TAC | ACT | GAA | CAT | TCT | TCG | GCC | GCT | TGG | GAC | ACT | ACT | 240 |
| Phe | Asn | Leu | Phe | Tyr | Thr | Glu | His | Ser | Ser | Ala | Ala | Trp | Asp | Thr | Thr | |
| | 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| CTT | CTA | GAA | CAA | CTG | TGC | ACT | GGT | CTG | CAA | CAG | CAA | CTG | GAC | CAT | CTG | 288 |
| Leu | Leu | Glu | Gln | Leu | Cys | Thr | Gly | Leu | Gln | Gln | Gln | Leu | Asp | His | Leu | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| GAC | ACT | TGC | CGT | GGC | CAG | GTT | ATG | GGT | GAA | GAA | GAC | TCT | GAA | CTG | GGT | 336 |
| Asp | Thr | Cys | Arg | Gly | Gln | Val | Met | Gly | Glu | Glu | Asp | Ser | Glu | Leu | Gly | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| AAC | ATG | GAT | CCG | ATC | GTT | ACT | GTT | AAA | AAA | TAT | TTC | CAG | GGT | ATC | TAC | 384 |
| Asn | Met | Asp | Pro | Ile | Val | Thr | Val | Lys | Lys | Tyr | Phe | Gln | Gly | Ile | Tyr | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| GAC | TAC | CTG | CAG | GAA | AAA | GGT | TAC | TCT | GAC | TGC | GCT | TGG | GAA | ATC | GTA | 432 |
| Asp | Tyr | Leu | Gln | Glu | Lys | Gly | Tyr | Ser | Asp | Cys | Ala | Trp | Glu | Ile | Val | |
| | | 130 | | | | 135 | | | | | 140 | | | | | |
| CGC | GTT | GAA | ATG | ATG | CGG | GCC | CTG | ACT | GTG | TCG | ACT | ACT | CTG | CAA | AAA | 480 |
| Arg | Val | Glu | Met | Met | Arg | Ala | Leu | Thr | Val | Ser | Thr | Thr | Leu | Gln | Lys | |
| | | | | | 150 | | | | | 155 | | | | | 160 | |
| CGG | TTA | ACT | AAA | ATG | GGT | GGT | GAC | CTG | AAT | TCT | CCG | | | | | 516 |
| Arg | Leu | Thr | Lys | Met | Gly | Gly | Asp | Leu | Asn | Ser | Pro | | | | | |
| | | | | 165 | | | | | | 170 | | | | | | |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: amino acid sequence of a mature OvIFNtau protein

~~(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:~~

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Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
 1           5           10           15
Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
          20           25           30
Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
          35           40           45
Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
50           55           60
Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
65           70           75           80
Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu
          85           90           95
Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
          100          105          110
Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
          115          120          125
Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
          130          135          140
Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
          145          150          155          160
Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
          165          170

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: synthetic nucleotide sequence encoding a mature human interferon-tau protein, HuIFNtau1.

~~(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:~~

TGTGACTTGT CTCAAAACCA CGTTTTGGTT GGTAGAAAGA ACTTAAGACT ACTAGACGAA

| | |
|---|-----|
| ATGAGACGTC TATCTCCACG CTTCTGTCTA CAAGACAGAA AGGACTTCGC TTTGCCTCAG | 120 |
| GAAATGGTTG AAGGTGGCCA ACTACAAGAA GCTCAAGCGA TATCTGTTTT GCACGAAATG | 180 |
| TTGCAACAAA GCTTCAACTT GTTCCACACC GAACACTCTT CGGCCGCTTG GGACACCACC | 240 |
| TTGTTGGAAC AGCTCAGAAC CGGTTTGCAC CAACAATTGG ACAACTTGGA TGCATGTTTG | 300 |
| GGTCAAGTTA TGGGTGAAGA AGACTCTGCT CTCGGGAGAA CCGGTCCAAC GCTAGCTTTG | 360 |
| AAGAGATACT TCCAAGGTAT CCACGTTTAC TTGAAGGAAA AGGGTTACTC TGACTGTGCT | 420 |
| TGGGAAACCG TGCCTCTAGA AATCATGCGT AGCTTCTCTT CTTTGATCAG CTTGCAAGAA | 480 |
| AGATTACGTA TGATGGACGG TGACTTGTCG AGCCCA | 516 |

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: amino acid sequence for a mature HuIFNtau protein, HuIFNtau1.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Asp | Leu | Ser | Gln | Asn | His | Val | Leu | Val | Gly | Arg | Lys | Asn | Leu | Arg | 1 | 5 | 10 | 15 |
| Leu | Leu | Asp | Glu | Met | Arg | Arg | Leu | Ser | Pro | Arg | Phe | Cys | Leu | Gln | Asp | 20 | 25 | 30 | |
| Arg | Lys | Asp | Phe | Ala | Leu | Pro | Gln | Glu | Met | Val | Glu | Gly | Gly | Gln | Leu | 35 | 40 | 45 | |
| Gln | Glu | Ala | Gln | Ala | Ile | Ser | Val | Leu | His | Glu | Met | Leu | Gln | Gln | Ser | 50 | 55 | 60 | |
| Phe | Asn | Leu | Phe | His | Thr | Glu | His | Ser | Ser | Ala | Ala | Trp | Asp | Thr | Thr | 65 | 70 | 75 | 80 |
| Leu | Leu | Glu | Gln | Leu | Arg | Thr | Gly | Leu | His | Gln | Gln | Leu | Asp | Asn | Leu | 85 | 90 | 95 | |
| Asp | Ala | Cys | Leu | Gly | Gln | Val | Met | Gly | Glu | Glu | Asp | Ser | Ala | Leu | Gly | 100 | 105 | 110 | |
| Arg | Thr | Gly | Pro | Thr | Leu | Ala | Leu | Lys | Arg | Tyr | Phe | Gln | Gly | Ile | His | 115 | 120 | 125 | |
| Val | Tyr | Leu | Lys | Glu | Lys | Gly | Tyr | Ser | Asp | Cys | Ala | Trp | Glu | Thr | Val | 130 | 135 | 140 | |
| Arg | Leu | Glu | Ile | Met | Arg | Ser | Phe | Ser | Ser | Leu | Ile | Ser | Leu | Gln | Glu | 145 | 150 | 155 | 160 |
| Arg | Leu | Arg | Met | Met | Asp | Gly | Asp | Leu | Ser | Ser | Pro | | | | | 165 | 170 | | |

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 516 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: HuIFNtau3, mature no leader sequence

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..516

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|---|-----|
| TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC AGC CAG AAC CTC AGG | 48 |
| Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Ser Gln Asn Leu Arg | |
| 1-----5-----10-----15 | |
| CTC CTG GGC CAA ATG AGG AGA CTC TCC CTT CGC TTC TGT CTG CAG GAC | 96 |
| Leu Leu Gly Gln Met Arg Arg Leu Ser Leu Arg Phe Cys Leu Gln Asp | |
| 20-----25-----30 | |
| AGA AAA GAC TTC GCT TTC CCC CAG GAG ATG GTG GAG GGT GGC CAG CTC | 144 |
| Arg Lys Asp Phe Ala Phe Pro Gln Glu Met Val Glu Gly Gly Gln Leu | |
| 35-----40-----45 | |
| CAG GAG GCC CAG GCC ATC TCT GTG CTC CAC GAG ATG CTC CAG CAG AGC | 192 |
| Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser | |
| 50-----55-----60 | |
| TTC AAC CTC TTC CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC | 240 |
| Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr | |
| 65-----70-----75-----80 | |
| CTC CTG GAG CAG CTC CGC ACT GGA CTC CAT CAG CAG CTG GAT GAC CTG | 288 |
| Leu Leu Glu Gln Leu Arg Thr Gly Leu His Gln Gln Leu Asp Asp Leu | |
| 85-----90-----95 | |
| GAT GCC TGC CTG GGG CAG GTG ACG GGA GAG GAA GAC TCT GCC CTG GGA | 336 |
| Asp Ala Cys Leu Gly Gln Val Thr Gly Glu Glu Asp Ser Ala Leu Gly | |
| 100-----105-----110 | |
| AGA ACG GGC CCC ACC CTG GCC ATG AAG AGG TAT TTC CAG GGC ATC CAT | 384 |
| Arg Thr Gly Pro Thr Leu Ala Met Lys Arg Tyr Phe Gln Gly Ile His | |
| 115-----120-----125 | |
| GTC TAC CTG AAA GAG AAG GGA TAT AGT GAC TGC GCC TGG GAA ATT GTC | 432 |
| Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val | |
| 130-----135-----140 | |
| AGA CTG GAA ATC ATG AGA TCC TTG TCT TCA TCA ACC AGC TTG CAC AAA | 480 |
| Arg Leu Glu Ile Met Arg Ser Leu Ser Ser Ser Thr Ser Leu His Lys | |
| 145-----150-----155-----160 | |
| AGG TTA AGA ATG ATG GAT GGA GAC CTG AGC TCA CCT | 516 |
| Arg Leu Arg Met Met Asp Gly Asp Leu Ser Pro | |
| 165-----170 | |

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Asp | Leu | Ser | Gln | Asn | His | Val | Leu | Val | Gly | Ser | Gln | Asn | Leu | Arg |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| <hr/> | | | | | | | | | | | | | | | |
| Leu | Leu | Gly | Gln | Met | Arg | Arg | Leu | Ser | Leu | Arg | Phe | Cys | Leu | Gln | Asp |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Arg | Lys | Asp | Phe | Ala | Phe | Pro | Gln | Glu | Met | Val | Glu | Gly | Gly | Gln | Leu |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Gln | Glu | Ala | Gln | Ala | Ile | Ser | Val | Leu | His | Glu | Met | Leu | Gln | Gln | Ser |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Phe | Asn | Leu | Phe | His | Thr | Glu | His | Ser | Ser | Ala | Ala | Trp | Asp | Thr | Thr |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| <hr/> | | | | | | | | | | | | | | | |
| Leu | Leu | Glu | Gln | Leu | Arg | Thr | Gly | Leu | His | Gln | Gln | Leu | Asp | Asp | Leu |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Asp | Ala | Cys | Leu | Gly | Gln | Val | Thr | Gly | Glu | Glu | Asp | Ser | Ala | Leu | Gly |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Arg | Thr | Gly | Pro | Thr | Leu | Ala | Met | Lys | Arg | Tyr | Phe | Gln | Gly | Ile | His |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Val | Tyr | Leu | Lys | Glu | Lys | Gly | Tyr | Ser | Asp | Cys | Ala | Trp | Glu | Ile | Val |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Arg | Leu | Glu | Ile | Met | Arg | Ser | Leu | Ser | Ser | Ser | Thr | Ser | Leu | His | Lys |
| 145 | | | | 150 | | | | | 155 | | | | | | 160 |
| Arg | Leu | Arg | Met | Met | Asp | Gly | Asp | Leu | Ser | Ser | Pro | | | | |
| | | | 165 | | | | | 170 | | | | | | | |

IT IS CLAIMED:

1. Use of tau-interferon (IFN τ) for the manufacture of a medicament for treating an autoimmune disease in a mammalian subject by administering to the subject a
5 pharmaceutically effective amount of said medicament.

2. ~~The use according to claim 1, wherein said autoimmune disease is multiple sclerosis.~~

10 3. The use according to claim 1, wherein said IFN τ is selected from the group consisting of ovine IFN τ , bovine IFN τ , goat IFN τ , ox IFN τ , rat IFN τ , mouse IFN τ and human IFN τ .

15 4. The use according to claim 3, wherein said IFN τ is ovine IFN τ (OvIFN τ).

5. The use according to claim 4, wherein said OvIFN τ has the sequence represented as SEQ ID NO:2.

20 6. The use according to claim 3, wherein said IFN τ is human IFN τ (HuIFN τ).

7. The use according to claim 6, wherein said HuIFN τ has the sequence represented as SEQ ID NO:4.

25 8. The use according to claim 1, wherein said IFN τ is a recombinantly produced IFN τ .

9. The use according to claim 1, wherein said administering is done by injection.

30 10. The use according to claim 1, wherein said administering is done by oral administration and said medicament is ingested by said subject.

11. The use according to claim 1, wherein said mammalian subject is a human subject.

12. The use according to claim 1, wherein said pharmaceutically effective amount includes between about 1×10^5 and about 1×10^8 units of IFN γ per day.

13. The use according to claim 12, wherein said pharmaceutically effective amount includes between about 1×10^6 and about 1×10^7 units of IFN γ per day.

14. The use according to claim 1, wherein said administering further includes administering a second autoimmune disease treatment agent.

15. The use according to claim 14, wherein said second agent is a corticosteroid drug.

16. Use of tau-interferon (IFN τ) for the manufacture of a medicament for treating a disease responsive to treatment by IFN τ by orally administering said medicament to a mammalian subject.

17. The use according to claim 16, wherein said disease is an autoimmune disease.

18. The use according to claim 17, wherein said autoimmune disease is multiple sclerosis.

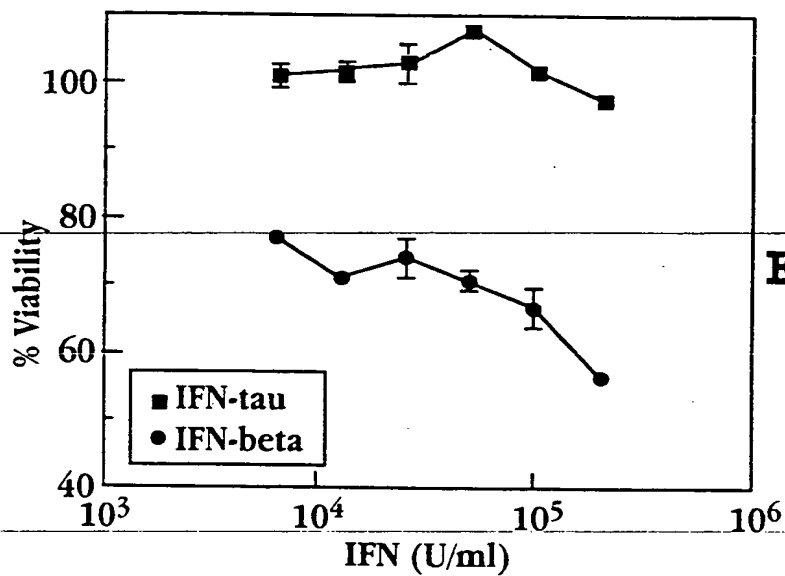
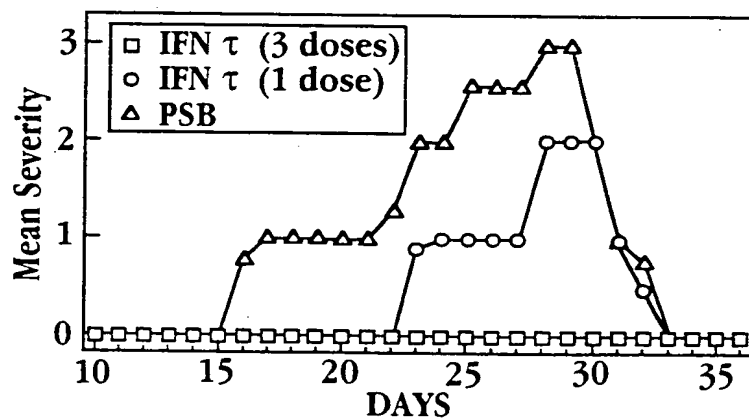
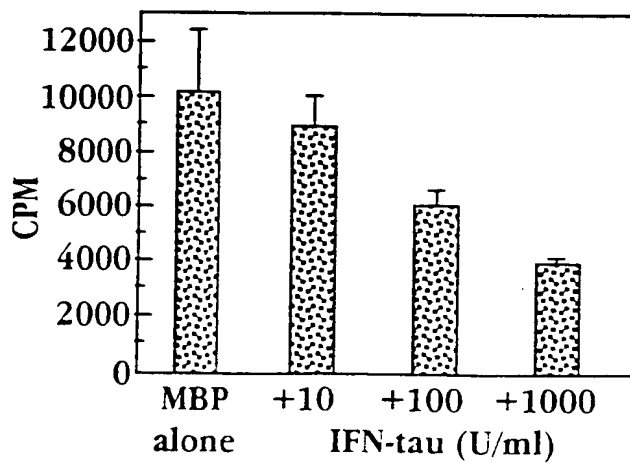
19. The use according to claim 16, wherein said IFN τ is selected from the group consisting of ovine IFN τ , bovine IFN τ , goat IFN τ , ox IFN τ , rat IFN τ , mouse IFN τ and human IFN τ .

20. The use according to claim 19, wherein said IFN τ is human IFN τ (HuIFN τ).

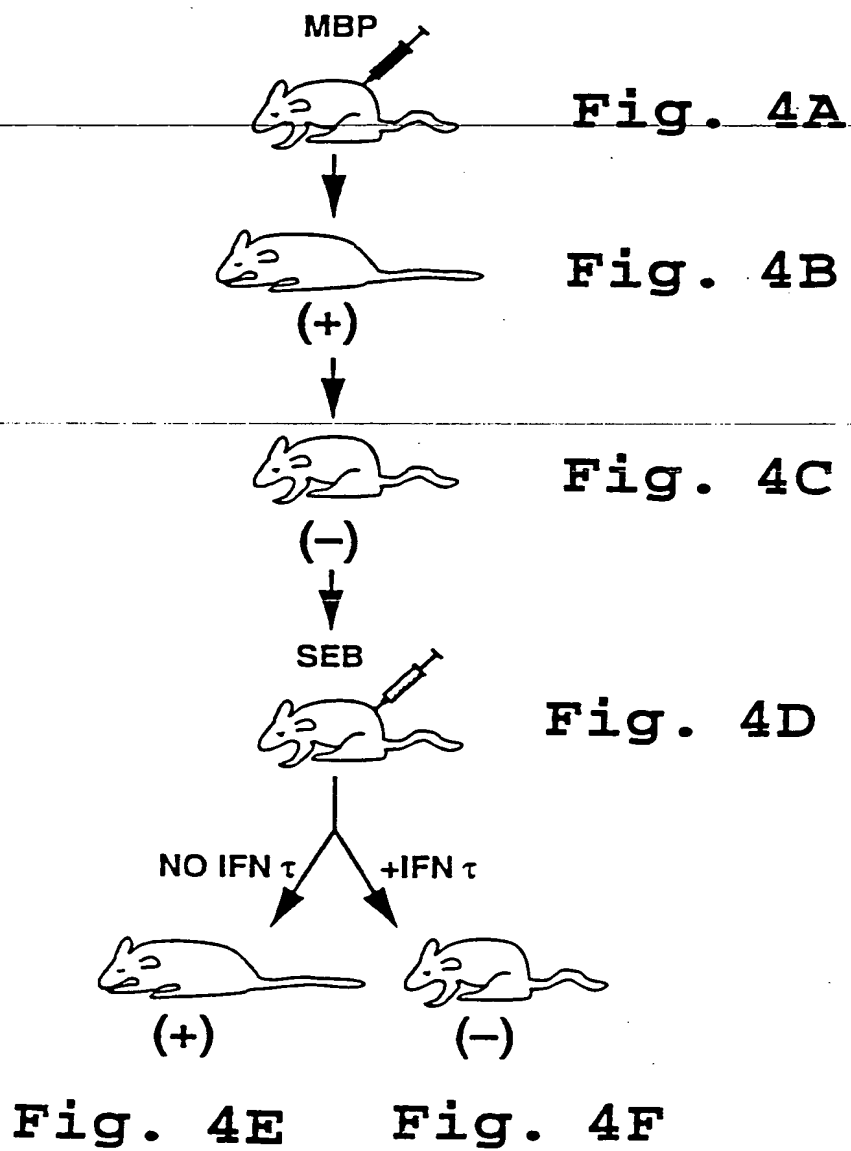
21. The use according to claim 16, wherein said IFN τ is a recombinantly produced IFN τ .

22. The use according to claim 16, wherein said subject is a human subject.

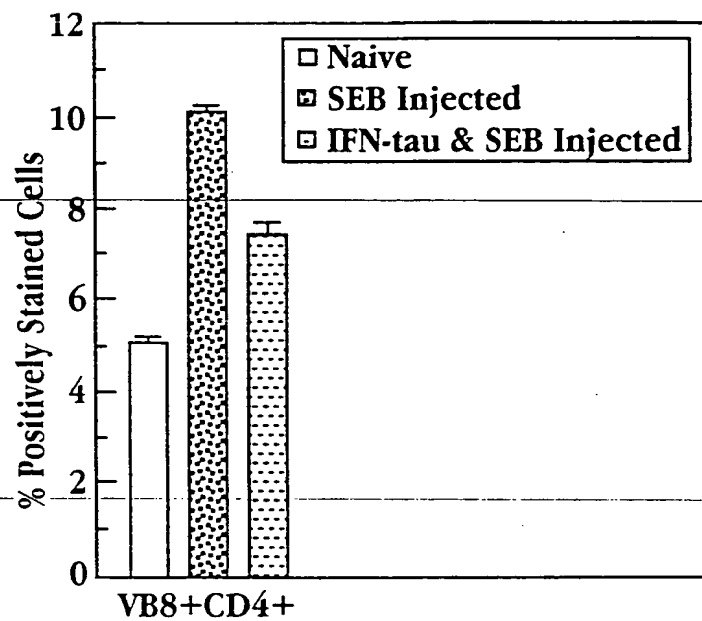
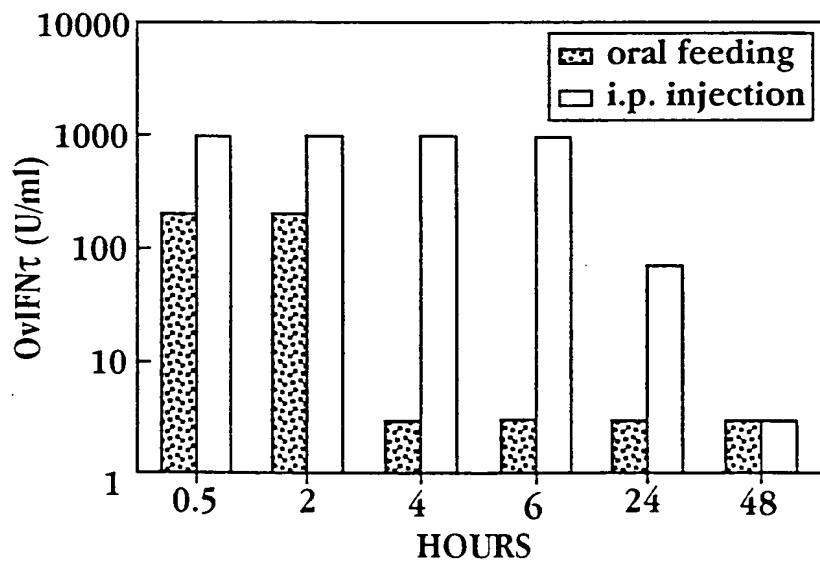
1/7

**Fig. 1****Fig. 2****Fig. 3**

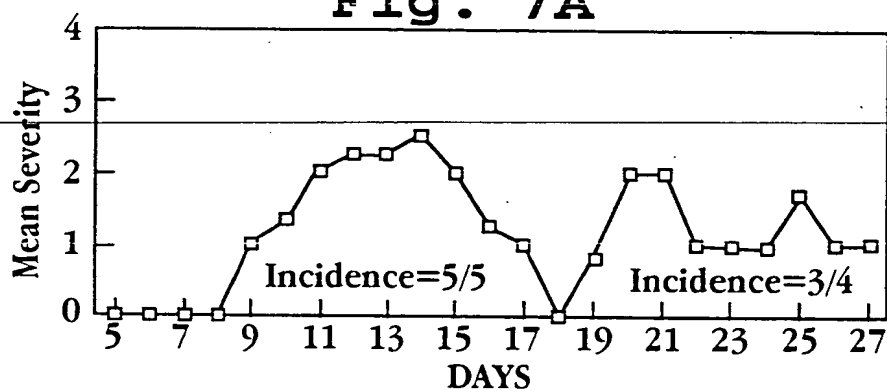
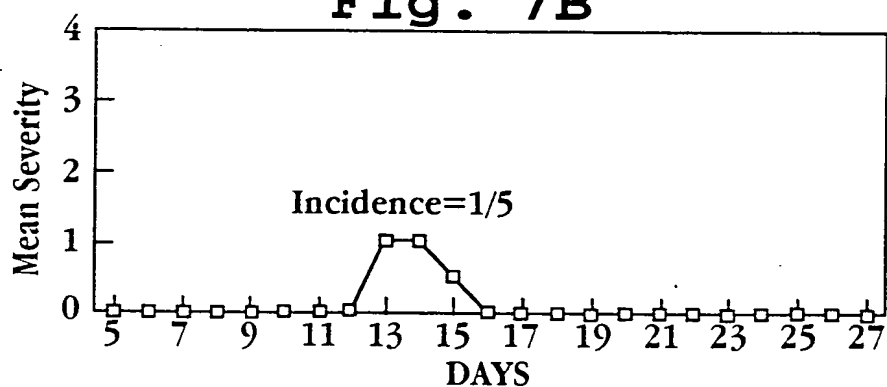
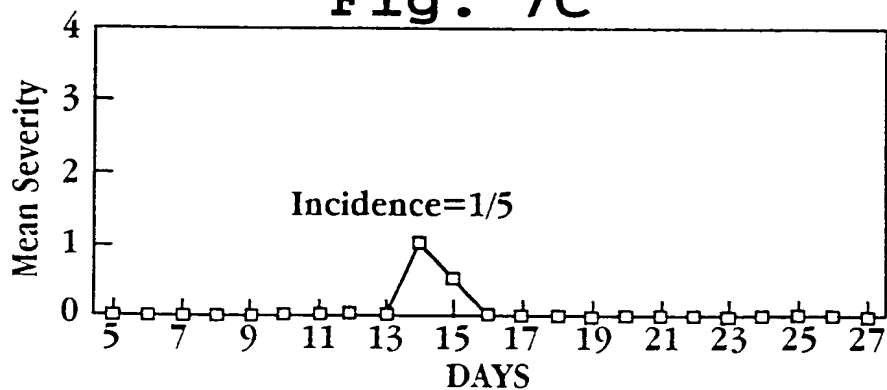
2/7



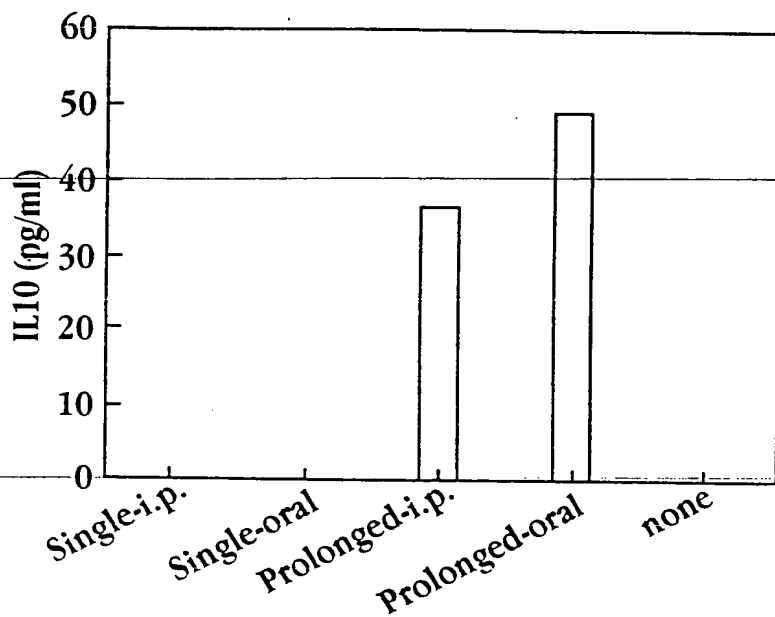
3/7

**Fig. 5****Fig. 6**

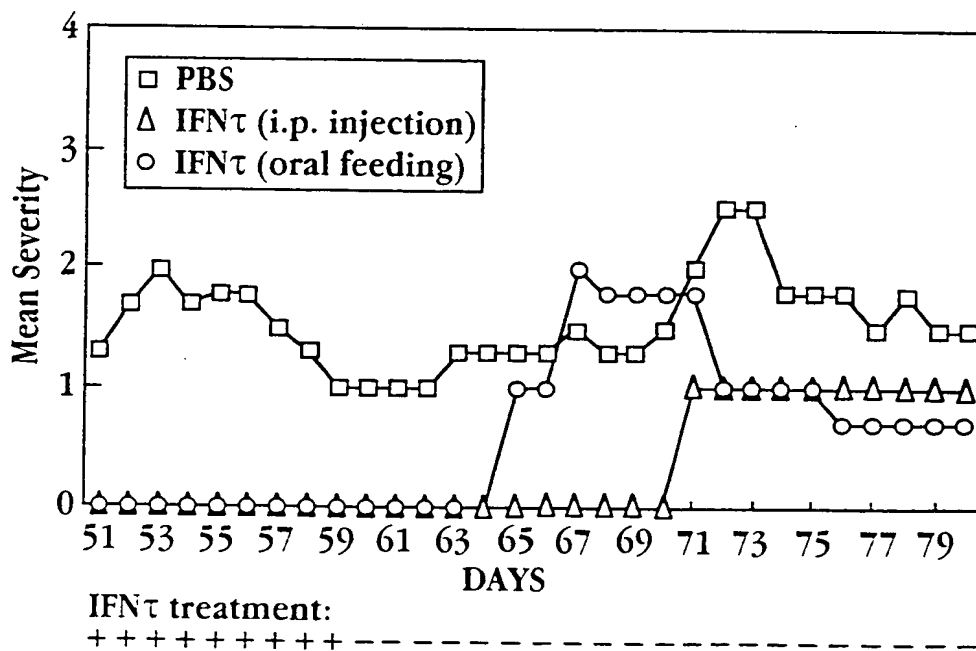
4/7

Fig. 7A**Fig. 7B****Fig. 7C**

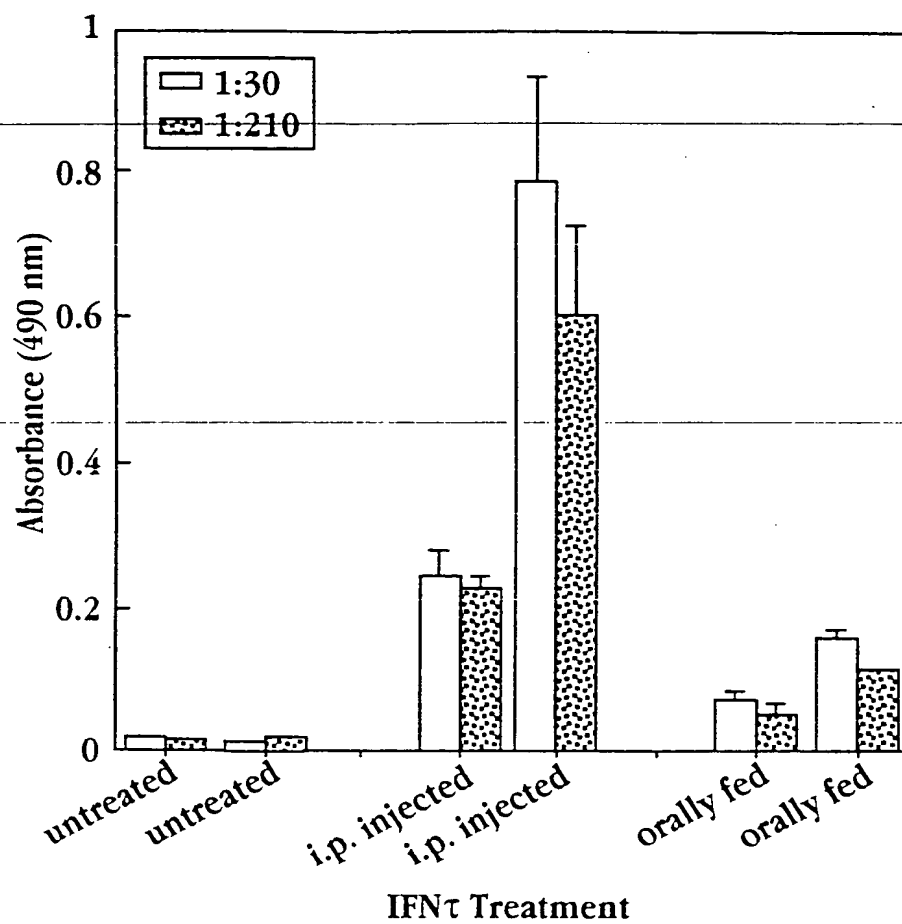
6/7



IFNτ Treatment

Fig. 9**Fig. 10**

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**Fig. 11**

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/03472

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/21 C07K14/555 C12N15/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | <p>FASEB JOURNAL FOR EXPERIMENTAL BIOLOGY, vol. 9, no. 4, 10 March 1995, BETHESDA, MD US, page A1025 XP002010951 J.M. SOOS ET AL: "The novel Type I Interferon Tau development and superantigen reactivation of experimental allergic encephalomyelitis in mice without associated toxicity" & EXPERIMENTAL BIOLOGY 95 PART II, 9 - 13 April 1995, ATLANTA ; GEORGIA ,USA., see abstract 5940</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p> | 1-5,8 |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

14 August 1996

Date of mailing of the international search report

28.08.96

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Authorized officer

Le Cornec, N

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/03472

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
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| WO-A-9410313 | 11-05-94 | AU-B- 5444994 | 24-05-94 |
| | | CN-A- 1090510 | 10-08-94 |
| | | EP-A- 0669981 | 06-09-95 |
| | | JP-T- 8505047 | 04-06-96 |